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FERNANDA MATEUS DAMIN

INVESTIGAÇÃO DO CONTEÚDO DE ÁCIDOS CLOROGÊNICOS, ÁCIDO CAFEICO E RUTINA EM FRUTAS E HORTALIÇAS COMERCIALIZADAS NO BRASIL

SCREENING OF CHLOROGENIC ACIDS, CAFFEIC ACID AND RUTIN CONTENT IN FRUITS AND VEGETABLES COMMERCIALIZED IN BRAZIL

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Dissertação de Mestrado apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Ciência de Alimentos.

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Orientadora: Profa Dra Helena Teixeira Godoy

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A ata de defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica da aluna.

“A verdadeira sabedoria consiste em se conhecer a própria ignorância.”

(Sócrates)

“Viver é a coisa mais rara do mundo. A maioria das pessoas apenas existe.”

(Oscar Wilde)

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RESUMO

Frutas e hortaliças são fontes importantes de macro e micronutrientes. Além disso, a presença de compostos bioativos representa um atrativo adicional destas duas classes de alimentos para os consumidores, que vem cada vez mais buscando um maior valor nutritivo e efeitos benéficos a saúde em suas dietas. Os compostos bioativos, que incluem os compostos fenólicos e, dentre estes - a rutina, os ácidos cafeico e clorogênicos - se caracterizam como substâncias não-nutrientes que possuem uma ação metabólica ou fisiológica específica. Os ácidos clorogênicos são um grupo de compostos fenólicos presentes em vários alimentos e bebidas como café, chás, frutas e vegetais e se caracterizam por serem ésteres de ácidos hidroxicinâmicos com o ácido quínico. Seus efeitos benéficos comprovados variam desde ação antioxidante, antimicrobiana, antibactericida, antiviral, anti-inflamatória, redução de estresse oxidativo e prevenção do risco de doenças crônicas como câncer, doenças coronárias, diabetes e obesidade. Levando em consideração o efeito potencial dos ácidos clorogênicos e outros compostos bioativos em relação à benefícios a saúde, é importante ter conhecimento do seu conteúdo em frutas e verduras com o intuito de identificar fontes potenciais que possam ser incluídas na dieta e, inclusive, agregar valor à cultivos ainda pouco explorados. Sendo assim, o projeto tem como objetivo identificar e quantificar ácidos clorogênicos, ácido caffeico e rutina presentes em frutas e hortaliças comercializadas no Brasil. As amostras foram adquiridas em triplicata de três produtores diferentes e os compostos de interesse foram quantidicados através de cromatografia líquida de alta eficiência. Em relação à rutina, as amostras que se destacara foram o coentro, umbu, aspargo, noni, amora, marmelo e cereja. Entre as frutas, 67% apresentaram valores quantificáveis de um ou mais isômero de ácidos clorogênicos e cafeico, sendo que o mirtilo e a pitaya foram as melhores fontes de ácido cafeico. Quando considerada a somatória de ácidos cafeoilquínicos (3-CQA, 4-CQA e 5-CQA), os destaques foram o morango, cereja, marmelo e amora, já para os dicafeoilquínicos (3,4-DQA, 3,5-DQA e 4,5-DQA) apresenta-se a kinkan, maracujá, e granadilha. Com relação às hortaliças avaliadas, as que se destacaram quanto ao somatório dos teores de ácidos monocafeoilquínicos são a couve-manteiga, o almeirão e a alface-roxa, enquanto para o somatório dos dicafeoilquínicos estão o louro, a mostarda e o salsão. Para o ácido cafeico, o orégano, a sálvia e o alecrim se mostraram com as quantidades mais significativas. Várias das espécies estudadas não possuíam estudos em relação à presença dos seis isômeros de ácidos clorogênicos, ácido cafeico e rutina, demonstrando neste trabalho uma gama considerável de novas informações acerca destes compostos bioativos, bem como a identificação de novas fontes.

ABSTRACT

Fruits and vegetables are regarded as important sources of both macro and micronutrients. In addition, the presence of bioactive compounds creates an additional interest to these two classes of food in relation to consumers who are, increasingly, seeking a better nutritional value and beneficial health effects in their diets. Bioactive compounds, which include phenolic compounds and among them - rutin, caffeic and chlorogenic acids - are characterized as non-nutrients that have a specific metabolic or physiological action. Chlorogenic acids are a group of phenolic compounds present in various foods and beverages such as coffee, teas, fruits and vegetables and are characterized by being esters of hydroxycinnamic acids with quinic acid. Their beneficial effects range from antioxidant, antimicrobial, antibacterial, antiviral, and anti-inflammatory activity, to oxidative stress reduction and risk prevention of chronic diseases such as cancer, coronary heart disease, diabetes and obesity. Taking into account the potential effect of chlorogenic acids and other bioactive compounds on health benefits, it is important to be aware of their content in fruits and vegetables in order to identify potential sources that can be included in the diet and even add value to crops not yet explored. Thus, this research aims to identify and quantify chlorogenic acids, caffeic acid and rutin present in fruits and vegetables commercialized in Brazil. The samples were obtained in triplicate from three different producers and the compounds of interest were quantified by high performance liquid chromatography. Regarding the results of rutin, the samples that stood out were coriander, umbu, asparagus, noni, blackberry, quince and cherry. Among fruits, 67% presented quantifiable values of one or more isomers of chlorogenic and caffeic acids, and blueberry and pitaya were the best sources of caffeic acid. When the sum of caffeine-like acids (3-CQA, 4-CQA and 5-CQA) was considered, the highlights were strawberry, cherry, quince and blackberry, while for the dicafeoilquinic acids (3,4-DQA, 3,5-DQA and 4,5-DQA) the highest values were found in kinkan, passion fruit, and wildflower. Regarding the evaluated vegetables, the ones that stood out for the sum of the monocafeoilquínicos acids contents are cauliflower, chicory and purple lettuce, whereas for the sum of the dicafeoilquínicos were laurel, mustard and celery. For caffeic acid, oregano, sage, and rosemary showed the most significant amounts. Several of the studied species had no previous data regarding the presence of the six isomers of chlorogenic acids, caffeic acid and rutin, demonstrating in this work a considerable range of new information about these bioactive compounds, as well as the identification of new sources.

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INTRODUÇÃO GERAL

Em virtude de todo o conhecimento disseminado em relação à composição e os benefícios do consumo de frutas e hortaliças, sua ingestão não é mais procurada apenas devido à atributos sensoriais ou preferências dos consumidores, mas também pelos efeitos positivos na saúde. Pessoas que buscam uma vida mais saudável procuram incluir alimentos dessas classes em sua dieta, já que vários estudos os correlacionam com a diminuição do risco de doenças crônicas como diabetes, câncer e obesidade (KREMR et al., 2015; NAVEED et al., 2018; SIRIAMORNPUN; KAEWSEEJAN, 2017).

Além de serem fonte de diversos macro e micronutrientes, as frutas e hortaliças possuem várias classes de compostos bioativos, como os compostos fenólicos, carotenoides e tocoferóis. Dentre os compostos fenólicos destacam-se várias classes, como os flavonoides – dos quais a rutina é um representante -, os ácidos clorogênicos e o ácido cafeico. Essas substâncias se caracterizam por possuírem funções fisiológicas ou metabólicas específicas as quais já foram relacionadas à redução do estresse oxidativo e prevenção de diversas doenças crônicas (ALONSO-CASTRO; DOMÍNGUEZ; GARCÍA-CARRANCÁ, 2013; BAO et al., 2018; GANESHPURKAR; SALUJA, 2016; JESZKA-SKOWRON; STANISZ; DE PEÑA, 2016).

Nesse contexto, os ácidos clorogênicos se enquadram na classe de compostos fenólicos, sendo que as principais fontes desses compostos já descritas na literatura científica são o café e chás (BUDRYN; ZACZYŃSKA; ORACZ, 2016; CRAIG et al., 2016; DA SILVEIRA et al., 2016). Os benefícios à saúde, relacionados a essa classe vêm sendo estudado por diversos pesquisadores que chegaram à conclusão de que há um impacto positivo na prevenção de doenças (HUANG et al., 2017b; MILLS et al., 2016; SISWANTO; OGURO; IMAOKA, 2017). Entretanto, não existem estudos extensos acerca do conteúdo de ácidos clorogênicos em diversas frutas e hortaliças, o que deixa em aberto um potencial de descoberta para novas fontes dessas substâncias.

O Brasil é um grande produtor de hortaliças e frutas, sendo que no caso das últimas, o país se encontra em terceiro lugar no panorama mundial de produção, estando atrás apenas da China e da Índia. As exportações de frutas ultrapassam a marca de 600 mil toneladas,, sendo que as espécies mais comercializadas com outros países são melão, manga, limão e banana, todos cultivos já estabelecidos pelos produtores brasileiros (SEBRAE, 2015). No entanto, a vasta biodiversidade do país, conta com uma grande quantidade de espécies vegetais que

ainda não tem seu potencial inteiramente explorado, o que gera falta de interesse em sua produção em virtude do baixo valor agregado (SCHIASSI et al., 2018).

A cromatografia é uma técnica de separação de substâncias que se baseia no transporte forçado de uma fase móvel (FM) contendo o analito através de uma fase estacionária (FE), as diferentes interações das substâncias da amostra com a FM e a FE resultam em migrações distintas dos compostos, permitindo assim sua separação (MALVIYA et al., 2010). A análise cromatográfica é dividida em vários tipos, sendo a cromatografia líquida de alta eficiência (HPLC) um método que usa solventes como fase móvel. (KAZAKEVICH; LOBRUTTO, 2006).

Devido a sua grande flexibilidade e sensibilidade, a HPLC é um dos métodos mais comuns para a análise de compostos fenólicos de matrizes de origem vegetal, ela tem sido utilizada na separação de várias classes como antocianinas, taninos, flavanóis, flavonóis, ácidos fenólicos, entre outros (CAPRIOTTI et al., 2014).

O conhecimento do conteúdo de compostos bioativos, como os ácidos clorogênicos, ácido cafeico e rutina em frutas e hortaliças ajuda a agregar valor e promover interesse em espécies que podem vir a ser fontes potenciais dessas substâncias, inclusive sugerindo alternativas às fontes tradicionais já conhecidas na ciência. Dessa forma, este trabalho tem como objetivo identificar e quantificar o ácido cafeico, rutina e isômeros de ácidos clorogênicos em frutas e hortaliças comercializadas no Brasil utilizando cromatografia líquida de alta eficiência (HPLC).

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OBEJTIVOS

OBJETIVO GERAL

Identificar e quantificar, através de cromatografia líquida de alta eficiência, seis isômeros de ácidos clorogênicos, ácido cafeico e rutina em amostras de frutas e hortaliças comercializadas no Brasil a fim de identificar fontes potenciais desses compostos.

OBJETIVOS ESPECÍFICOS

I) Implementar e validar uma metodologia de HPLC para quantificação de ácidos clorogênicos, ácido cafeico e rutina.

II) Determinar umidade, acidez e teor de sólidos solúveis para caracterizar as condições pós-colheita das amostras.

III) Identificar fontes potenciais de ácidos clorogênicos e ácido cafeico.

CAPÍTULO I: REVISÃO DA LITERATURA

1. PRODUÇÃO DE FRUTAS E HORTALIÇAS NO BRASIL

Frutas e hortaliças são presenças tradicionais na dieta humana, sendo que uma alimentação rica nesses constituintes é reconhecida por apresentar efeitos positivos em relação à saúde ao contribuir para a prevenção de diversas doenças degenerativas. Tal ação é atribuída à vários compostos presentes nas plantas como compostos fenólicos, tocoferóis, ácido ascórbico e outros (HUBER; HOFFMANN-RIBANI; RODRIGUEZ-AMAYA, 2009).

O Brasil se encontra entre os maiores produtores mundiais de hortaliças e frutas, sendo que, em relação às últimas, o país é o terceiro maior produtor do mundo. Sua produção supera os 40,0 milhões de toneladas em cerca de 2,5 milhões de hectares, dos quais totalizam 1,5% da área agriculturável brasileira, e geram aproximadamente 6 milhões de mempregos diretos. Entre as regiões do país que se dedicam à produção de frutas, o Nordeste, Sul e Sudeste são as que se destacam. Devido às várias diferenças climáticas, existe uma ampla gama de frutas produzidas no país, tais como: laranja, banana, uva, melão abacaxi, entre outras. Entretanto, ainda existe um numero expressivo de espécies nativas que permanecem inexploradas, principalmente frutas regionais (ANDRADE, 2017; SCHIASSI et al., 2018; SEBRAE, 2015).

Diferente do mercado nacional de frutas, que tem uma grande força de exportação (foram quase 200 mil toneladas exportadas em 2014 só levando em consideração o melão que foi a fruta mais exportada), as hortaliças apresentam uma produção mais voltada para o mercado interno onde estima-se que entre 55% e 60% do volume é comercializado em mercados atacadistas, que atingem vendas anuais de 15 milhões de toneladas de hortaliças (MAPA, 2017; SEBRAE, 2015)

Embora existam muitas evidências corroborando com as afirmações dos benefícios do consumo de frutas e hortaliças e uma crescente procura dos consumidores por produtos mais saudáveis, houve um aumento no consumo desses alimentos, porém ainda não atingindo patamares ideais (VAN DUYN; PIVONKA, 2000). A Organização Mundial da Saúde preconiza que o consumo diário de frutas e hortaliças deve ser de 400 g, contudo 50% da população brasileira falha em alcançar as orientações de consumo, o que aumenta o risco da população de desenvolver uma gama de doenças (HALL et al., 2009).

2. ÁCIDOS CLOROGÊNICOS

2.1. DEFINIÇÕES GERAIS

Os ácidos clorogênicos são um grupo de compostos fenólicos presentes em vários alimentos e bebidas como café, chás, frutas e vegetais (BRAHEM et al., 2017; MALDINI et al., 2016; NAVEED et al., 2018; WOJDYŁO et al., 2016). Essa classe é caracterizada por serem ésteres formados pelo ácido químico e um ácido trans-hidroxicinâmico, sendo que os ácidos hidroxicinâmicos mais comuns são o cafeico, o ferrúlico e o p-cumárico. Existem relatos da existência de isômeros na configuração cis, porém estes parecem se restringir à tecidos de plantas onde a forma trans do composto sofreu alta exposição a radiação ultra violeta (JAISWAL et al., 2014).

Inicialmente, o termo ácido clorogêncio foi usado como nomenclatura para um dos componentes mais abundantes no café que hoje é conhecido como ácido 5-cafeoilquínico (5-CQA), que pode ser visto na Figura 1 e, desde então, passou a designar a classe inteira desses compostos que incluem ácidos ferruloilquínicos, cumaroilquínicos, dicafeoilquínicos, bem como os vários isômeros de cada subgrupo (KREMR et al., 2015; MORISHITA; OHNISHI, 2001). Entre os isômeros dos ácidos cafeoilquínicos e dicafeoílquínicos (um ácido quínico ligado a dois ácidos cafeicos) os mais comuns são o ácido 5-cafeoilquínico (5-CQA), ácido 4-cafeoilquínico (4-CQA), ácido 3-cafeoilquínico (3-CQA), ácido 3,4-dicafeoilquínico (3,4-DQA), ácido3,5 dicafeoiquínico (3,5-DQA) e o ácido 4,5-dicafeoilquínico (4,5-DQA) (NAVEED et al., 2018).

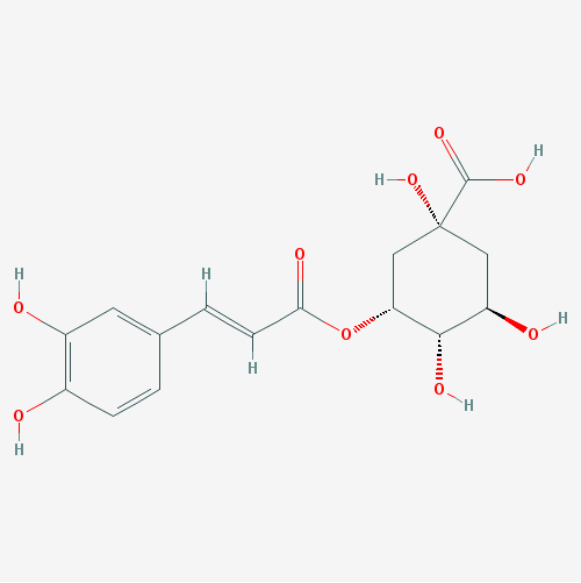

Figura 1 Estrutura do ácido 5-cafeoilquínico FONTE: PubChem

Análoga às outras classes de compostos fenólicos, os ácidos clorogênicos são tidos como produtos do metabolismo secundário das plantas com a funcionalidade de protegê-las do estresse gerado pelo ambiente (CRAIG et al., 2016). Dessa forma, essas moléculas apresentam funções bioativas como capacidade antioxidante, antimicrobiana, antibactericida, antiviral e anti-inflamatória que podem estar ligadas aos benefícios à saúde aos quais já foram relacionados (BAJKO et al., 2016; BUTIUK et al., 2016; PENG et al., 2015).

2.2. BENEFÍCIOS À SAÚDE

Os ácidos clorogênicos vêm sendo relacionados a efeitos benéficos à saúde por diversos estudos já publicados que ressaltam várias de suas propriedades como ação antioxidante (WONGSA; CHAIWARIT; ZAMALUDIEN, 2012), anti-diabética (BAO et al., 2018), anti-inflamatória (DOS SANTOS et al., 2006), anti-HIV (MCDOUGALL et al., 1998) e anti-carcinogênica (FENG et al., 2005). Esses compostos já evidenciaram a capacidade de modificar o metabolismo da glicose, podendo inclusive atuar em sua absorção (PENG et al., 2015; WANG; CLIFFORD; SHARP, 2008). Bao et al. (2018) constataram a ação dos ácidos clorogênicos em atenuar o estresse oxidativo e a inflamação na nefropatia diabética, atuando na proteção contra lesões renais diabéticas in vitro e in vivo. Hong et al. (2017) concluíram que esses compostos possuem efeitos benéficos no gerenciamento de disfunções auditivas sensorioneurais causadas por diabetes em estudo realizado com ratos. Ainda relacionado ao tratamento de diabetes, Sanchez et al. (2017) indicaram em seus resultados que os ácidos clorogênicos possuem múltiplos mecanismos de ação propícios para o desenvolvimento de tratamentos altamente eficientes contra doenças metabólicas como essa, principalmente por sua ação reguladora da glicose.

Foi reportado por alguns autores que os ácidos clorogênicos apresentarem influência no tratamento de doenças hepáticas. Evidências de que esses compostos protegem o fígado contra a formação de fibroses tanto induzidas por CCl4 quanto causadas por colestase foram demonstradas por Shi et al. (2016) e Wu et al. (2015). Já Zheng et al., (2015) demonstraram a capacidade desintoxicante do ácido clorogênico em feridas hepáticas causados pelo uso de paracetamol. Além dos demais efeitos ligados ao fígado, também existe dados relatando a capacidade de inibição da duplicação do vírus da Hepatite B (WANG et al. (2009).

O metabolismo lipídico e a interferência benéfica na absorção do colesterol também têm sido conectados a efeitos dos ácidos clorogênicos (ARANTES et al., 2016). Foi

demonstrado que essas substâncias inibiram a atividade hepática da glicose-6-fosfatase, diminuiram a esteatose hepática e melhoraram o perfil lipídico e absorção da glicose muscular esquelética (ONG; HSU; TAN, 2013). Wan et al. (2013) verificaram que os ácidos clorogênicos reduziram significativamente o colesterol total e LDL e levaram ao aumento do colesterol HDL. Já foi observada também a capacidade de inibir a biossíntese do colesterol além de apresentar potencial anti-obesidade em ratos com dietas hiperlipídicas (CHO et al., 2010). Gugliucci e Bastos (2009) concluiram que os ácidos clorogênicos aumentam a proteção da atividade da enzima paraoxonase, que participa do mecanismo de oxidação do LDL e HDL.

Adicionalmente, os ácidos clorogênicos foram correlacionados a efeitos antitumorais, com resultados relevantes contra o câncer de estômago, de cólon e até mesmo na repressão de fatores carcinogênicos relacionados (MATSUNAGA et al., 2002a; SHAO et al., 2015). Yan et al. (2017) apresentaram evidências de que esses compostos podem evitar a progressão do carcinoma hepatocelular e Siswanto, Oguro e Imaoka, (2017) concluíram que os mesmos foram capazes de inibir o crescimento de células cancerígenas no fígado de humanos. Foi observado também o efeito inibitório dos ácidos clorogênicos na hiperplasia prostática benigna em camundongos (HUANG et al., 2017b).

É possível ainda encontrar outras ações dos ácidos clorogênicos em relação a impactos benéficos à saúde como sua capacidade de proteger as células endoteliais do estresse oxidativo (JIANG et al., 2016), função antiglicante (BHATTACHERJEE; DATTA, 2015) e também o efeito de proteção dos neurônios contra o efeito tóxico do glutamato (MIKAMI; YAMAZAWA, 2015).

3. ÁCIDO CAFEICO

3.1. DEFINIÇÕES GERAIS

Os compostos fenólicos são substâncias isoladas das plantas e podem ser divididos em várias classes diferentes. Entre essas classes, existem os ácidos fenólicos, mais especificamente os ácidos hidroxicinâmicos e os ácidos hidroxibenzóicos (KHAN; MAALIK; MURTAZA, 2016). O ácido cafeico é um ácido hidroxicinâmico, derivado do catecol, e pode ser encontrado em várias espécies de plantas, principalmente o café e chás como a erva mate, o chá verde e a flor de sabugueiro (MEINHART et al., 2017; MURTAZA et al., 2015)

A estrutura do ácido caféico pode ser vista na Figura 2

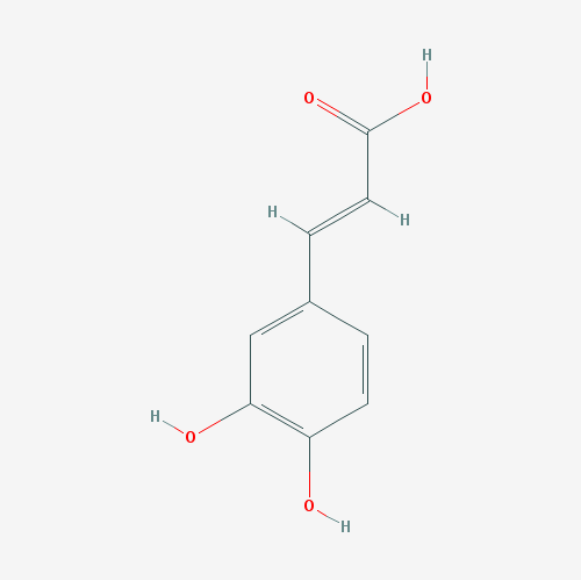

Figura 2 Estrutura do ácido cafeico FONTE: PubChem

3.2. BENEFÍCIOS À SAÚDE

O ácido cafeico é um composto que gera interesse por parte dos pesquisadores em virtude das várias atividades biológicas e farmacêuticas a ele atribuídas. Entre elas, inclui-se atividade anti-inflamaória, anti-aids e tividade antioxidante que está relacionada à sua estrutura molecular contendo um grupo catecol que é capaz de interagir com vários tipos de radicais oxidantes (FESEN et al., 1994; LARANJINHA; ALMEIDA; MADEIRA, 1994; MEDINA et al., 2012).

Kim et al. (2018) realizaram um in vivo onde três grupos de ratos foram avaliados, um submetido a uma dieta com baixo teor de lipídeos, outro com uma dieta hiperlipídica e o terceiro também com uma dieta hiperlipídica, porém consumindo 50 mg/Kg/dia de áído cafeico. Os resultaram demonstratam que a ingestão de ácido cafeico diminuiu a hesteatose hepática caudasa pela dieta com altos teores de lipídeos além de impactar positivamente a sensibilidade à insulina e intolerância à glicose.

Ainda relacionando o ácido clorogênico com benefícios à saúde, Basu Mallik et al. (2016) avaliaram a influência desse composto no comportamento depressivo causado por lipopolissacarídeos em ratos. O estudo concluiu que a ingestão de ácido cafeico atenuou o comportamento depressivo e a neuroinflamação causada pelos lipopolissacarídeos, sendo que eles sugerem que estudos futuros devem ser realizados para entender o mecanismo que leva a tal ação.

4. RUTINA

4.1. DEFINIÇÕES GERAIS

Os compostos fenólicos são compostos produzidos pelo metabolismo secundário das plantas como uma forma de mecanismo de defesa. Essas substâncias podem ser divididas em várias classes, sendo a classe flavonoides uma delas (EL GHARRAS, 2009). Os flavonoides se caracterizam por possuírem três anéis, sendo dois fenólicos, e podem ser separados em sete grupos de acordo com os elementos ligados a eles: flavonas, flavanonas, flavononóis, isoflavonas, flavanóis, flavonóis e antocianinas. Esses compostos estão amplamente dispersos entre as plantas, sendo que mais de 2000 exemplares já foram identificados (IGNAT; VOLF; POPA, 2011; LU; XIAO; ZHANG, 2013).

Nesse contexto, a rutina é um composto fenólico que pertence ao grupo dos flavonóis, formado por uma molécula de quercetina com uma ramnose ligada ao carbono três (GHORBANI, 2017) como pode ser visto na Figura 3. Ela é sintetizada através da via do fenilpropanoide, onde há a transformação de fenilalanina em 4-cumaroil-coenzima A, seguida de ação enzimática (FAGGIO et al., 2017; SELEEM; PARDI; MURATA, 2017). É um dos flavonoides de maior importância na indústria farmacêutica, estando presente em formulações medicinais e terapêuticas patenteadas no mundo todo (CHUA, 2013; GULLÓN et al., 2017; SHARMA et al., 2013).

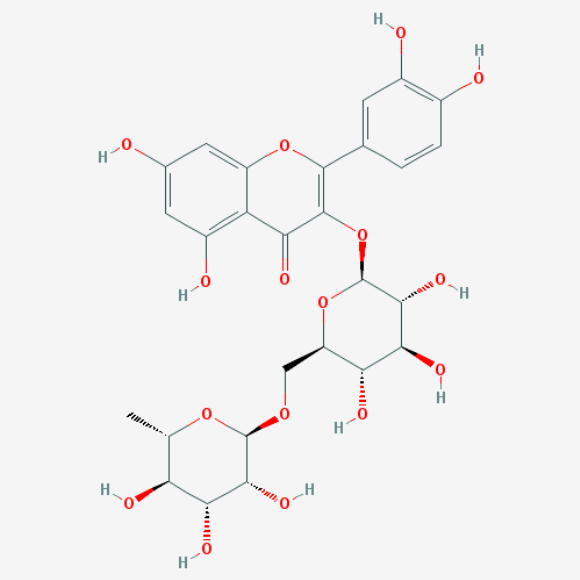

Figura 3 Estrutura da rutina FONTE: PubChem

A rutina é frequentemente encontrada em alimentos de origem vegetal, como frutas, hortaliças e grãos (ATANASSOVA; BAGDASSARIAN, 2009). Alguns exemplos já descritos na literatura são salsa, framboesa (PAVLOVIĆ et al., 2016; YILDIZ et al., 2008) açaí, ameixa roxa, noni, banana, laranja, goiaba (AMIR et al., 2013; GARZÓN et al., 2017; LIN et al., 2014; OBOH et al., 2015; PANDY et al., 2014), manjericão, almeirão (DALAR; KONCZAK, 2014; FRATIANNI et al., 2016), rabanete, cenoura (OBOH et al., 2015), lentilha (FRATIANNI et al., 2014a) e trigo sarraceno (KIM et al., 2005)

4.2. BENEFÍCIOS À SAÚDE

A rutina apresenta uma elevada capacidade antioxidante, além de possuir outras atividades biológicas importantes (ABARIKWU et al., 2017; HSU et al., 2009; PANCHAL et al., 2011; PRINCE; KAMALAKKANNAN, 2006). Estudos apontam que esse flavonoide tem efeito na melhora do estado hipoglicemiante de ratos diabéticos (HAO et al., 2012), e que a suplementação com esse composto foi eficiente na diminuição significativa dos níveis de glicose no sangue e na pressão arterial sistólica e diastólica (SATTANATHAN et al., 2011). Efeitos na redução da hipertrofia do miocárdio, aliviando a deposição de colágeno e o acúmulo de lipídios (HUANG et al., 2017a), bem como ação anti-inflamatória no tratamento de colite, peritonite e redução de edemas e citoquinas também foram reportado ao consumo de rutina na literatura (RABIŠKOVÁ et al., 2012; TORRES-RÊGO et al., 2016).

Estudos *in vivo* e *in vitro* demonstraram efeitos anticancerígenos desse flavonóide, indicando causar a redução no ciclo celular e induzir a apoptose em células cancerígenas (PERK et al., 2014), além de ação antiproliferativa e moduladora do carcinoma hepatocelular humano (KARAKURT, 2016), além do efeito quimiopreventivo e antitumorais *in vivo* (ALONSO-CASTRO; DOMÍNGUEZ; GARCÍA-CARRANCÁ, 2013; GONÇALVES et al., 2013). A rutina também foi relacionada com o alívio da aterosclerose (LI et al., 2018), assim como ações protetoras sobre a hepatotoxicidade (GELEN et al., 2017).

5. CROMATOGRAFIA LÍQUIDA DE ALTA EFICIÊNCIA

5.1. GENERALIDADES

Descoberta no início do século XX por um cientista russo chamado M. S. Tsweet, a cromatografia é uma das técnicas analíticas mais empregadas pelos cientistas quando o objetivo é separar, quantificar e identificar moléculas que componham uma determinada amostra (LANÇAS, 2009).

A cromatografia é uma técnica de separação de substâncias que se baseia no transporte forçado de uma fase móvel (FM) contendo o analito através de uma fase estacionária (FE), as diferentes interações das substâncias da amostra com a FM e a FE resultam em migrações distintas dos compostos, permitindo assim sua separação (MALVIYA et al., 2010).

A análise cromatográfica é dividida em vários tipos, quando levados em consideração o estado físico em que a FM e a FE se encontram, existe a cromatografia em coluna, na qual a FE se encontra dentro de um cilindro fixo, como é o caso da HPLC, ou a cromatografia plana, onde a FE é uma superfície plana, como uma folha de papel. Se a classificação for feita de acordo com os tipos de FM e FE existem três tipos principais, a cromatografia gasosa, a cromatografia líquida e a cromatografia de fluido supercrítico (KAZAKEVICH; LOBRUTTO, 2006).

Dentro da cromatografia líquida, que usa líquidos como FM e geralmente sólidos como FE, ainda há uma diversificação de acordo com o tipo de interação do analito com a superfície da fase estacionária e também em relação à polaridade das fases. São vários os tipos de cromatografia líquida: a de fase normal (FN), a de fase reversa (FR), exclusão por tamanho, bio-afinidade e troca iônica (KAZAKEVICH; LOBRUTTO, 2006).

Devido a sua grande flexibilidade, alta sensibilidade e ao emprego de temperaturas brandas durante a análise, a HPLC é um dos métodos mais comuns para a análise de compostos fenólicos de matrizes de origem vegetal, ela tem sido utilizada na separação de várias classes como antocianinas, taninos, flavanóis, flavonóis, ácidos fenólicos, entre outros (CAPRIOTTI et al., 2014).

5.2. FASE NORMAL E FASE REVERSA

A cromatografia líquida de alta eficiência de fase normal (FN) e fase reversa (FR) são as mais utilizadas quando o objetivo é a separação, identificação e quantificação de compostos fenólicos, sendo que estima-se que cerca de 90% de todas as separações analíticas são feitas por cromatografia líquida de fase reversa (COLLINS; BRAGA; BONATO, 2010).

Primeiro foi desenvolvida a técnica da fase normal que consiste em usar uma fase estacionária polar e uma fase móvel apolar. Em geral são usados solventes orgânicos como o hexano e o diclorometano como FM, sendo empregados em fases binárias juntamente com solventes polares. No entanto misturas binárias tem uma habilidade limitada para controlar a seletividade então podem ser usadas misturas terciarias ou até quaternárias (KAZAKEVICH; LOBRUTTO, 2006).

A separação na fase normal se dá pela retenção das moléculas pela fase estacionária através da adsorção, as moléculas mais polares tem maior afinidade pela FE, por isso são retidas por mais tempo (maior tempo de retenção) através de interações polares, enquanto as mais apolares apresentam maior afinidade com o solvente da FM, sendo então eluidas mais rapidamente. Em relação aos compostos fenólicos essa técnica não é muito utilizada, exceto quando a matriz possui compostos de alto peso molecular e baixa polaridade como é o caso dos taninos (COENTRÃO, 2005; ROBBINS et al., 2009).

A cromatografia em fase reversa (FR) é o oposto da fase normal, usa-se uma fase estacionária apolar e uma fase móvel polar. A FM é a melhor ferramenta para controlar o a retenção dos compostos analisados em FR, desse modo variações de composição do eluente, bem como tipo de modificador orgânico e pH são um conjunto de variáveis importantes para uma separação bem sucedida (COLLINS; BRAGA; BONATO, 2010).

As fases móveis mais utilizadas na FR, principalmente em relação aos compostos fenólicos, são misturas hidro-orgânicas, com metanol, tetrahidrofurano e acetonitrila, no entanto esses solventes apresentam forças diferentes, quanto mais forte o solvente, menor será a retenção do analito pela fase estacionária e, portanto devem ser selecionados de acordo com a necessidade da análise (ANTONIOLLI et al., 2015; XIE et al., 2011).

É recomendado adicionar um ácido à fase móvel para suprimir e existência de mais de uma forma da mesma substância (neutra e ionizada), pois cada forma terá um tempo de retenção diferente o que gera uma distorção no pico desse composto no cromatograma. Quando se trabalha num pH que está a uma unidade de diferença do pKa da substância, 90% das moléculas estarão na forma ionizada. Os ácidos mais comumente usados na análise de ácidos clorogênicos e compostos fenólicos em geral, são o fórmico e o acético, no entanto também há registros do uso do ácido trifluoracético, ácido fosfórico e ainda do ácido cítrico (FILHO et al., 2014; FU et al., 2008; HE et al., 2015; LIU et al., 2015; SCHIEBER; ULLRICH; CARLE, 2000; ŠERUGA; NOVAK; JAKOBEK, 2011; ZHANG et al., 2013).

Na cromatografia de fase reversa, as interações do analito com as fases móvel e estacionária se dá através de forças dispersivas (hidrofóbicas e de Van der Waals) que são mais fracas do que as interações polares da fase normal, dessa maneira a fase reversa apresente uma vantagem do ponto de vista energético, pois já que ela lida com forças de menores intensidades, é mais fácil distinguir variações e, portanto mais fácil de efetuar a separação (KAZAKEVICH; LOBRUTTO, 2006).

5.3. FASE ESTACIONÁRIA

A coluna é o local onde ocorre a separação propriamente dita e pode ser considerado o coração da cromatografia, portanto suas características são de suma importância para o sucesso da separação (GALEA; MANGELINGS; VANDER HEYDEN, 2015). Em geral elas são cilindros feitos de plástico ou de aço inoxidável e contém em seu interior a fase estacionária, podendo apresentar vários comprimentos e diâmetros.

Definir a qualidade de uma coluna é um parâmetro muito subjetivo, pois ele vai estar intimamente ligado à amostra a ser analisada e as condições usadas. Não existe fase estacionária ideal, cada uma apresenta pontos positivos e negativos para cada situação, no entanto, estabilidade o longo prazo e reprodutibilidade são características importantes na hora de selecionar uma coluna (LANÇAS, 2009). Quando se fala de fase estacionária para análise de ácidos clorogênicos e uma grande parte de outras classes de compostos fenólicos, colunas com empacotamento de sílica modificada com grupos apolares (C18) são as mais usadas, tornando-se praticamente uma regra (FRATIANNI et al., 2014a, 2016; ZHANG et al., 2015).

5.4. TIPOS DE DETECTORES

O detector é a parte mais sofisticada de um equipamento de HPLC, são eles que detectam as moléculas do analito presentes na fase móvel assim que saem da coluna e transformam em uma resposta (cromatograma) para serem interpretadas (CHRISTIE, 1992).

Vários princípios diferentes regem o funcionamento dos diversos detectores vendidos no mercado, sendo que não existe um detector universal, cada um tem suas vantagens e desvantagens para os múltiplos analitos que podem ser estudados (LANÇAS, 2009). Os detectores mais utilizados são os de índice de refração, fluorescência, espectrofotométricos e o espectrômetro de massa (MS), sendo que no caso dos compostos fenólicos o detector espectrofotométrico que atua na faixa UV-visível e o MS são os mais empregados (ANTONIOLLI et al., 2015; HOFMANN; NEBEHAJ; ALBERT, 2015; ISWALDI et al., 2013; WANG et al., 2008).

O funcionamento dos detectores espectrofotométricos se baseia na absorbância da luz pela amostra analisada. Uma luz com intensidade conhecida (I_0) é incidida a um certo comprimento de onda na amostra e então é medida a intensidade da radiação que é transmitida através da mesma (I). Essa relação permite o cálculo da concentração da amostra através da lei de Lambert-Beer (LANÇAS, 2009). Para que o uso desse detector seja eficiente, é necessário que a amostra de interesse apresente absorção na região do espectro, e já que

compostos com duplas ligações alternadas e anéis aromáticos tem uma boa absorção esse tipo de detector é o mais utilizado para analisar compostos fenólicos (principalmente o modelo de arranjo de diodos PDA).

O espectrômetro de massa é hoje o detector mais versátil para a análise de compostos orgânicos em misturas altamente complexas que permite além de identificar e quantificar compostos, elucidar também sua estrutura. Essa técnica se baseia na separação de íons no vácuo, obtendo uma relação massa/carga do composto. As moléculas analisadas precisam ser primeiramente ionizadas, sendo que existem várias técnicas diferentes com essa finalidade bem como diferentes tipos de analisadores de massa como o quadrupolo, o de tempo de voo e de aprisionamento de íons que são os mais usados (CAJKA; HAJSLOVA; MASTOVSKA, 2008). Cada vez mais o MS vem sendo utilizado para identificar e quantificar uma gama de compostos fenólicos, sua utilização só não é mais difundida em virtude de seu preço ainda ser muito alto em comparação com os demais, mas com a ampliação do número de usuários a tendência é a diminuição dos preços (LANÇAS, 2009).

5.5. APLICAÇÃO NA IDENTIFICAÇÃO E QUANTIFICAÇÃO DE COMPOSTOS FENÓLICOS

A pesquisa, qualitativa ou quantitativa, de compostos fenólicos em diversas matrizes naturais requer a utilização de métodos rápidos, sensíveis e confiáveis, sendo que diferentes técnicas podem ser utilizadas (XU et al., 2017). Quando o objetivo da análise está ligada a uma abordagem geral dos compostos presentes em uma amostra, os métodos espectrofotométricos podem ser utilizados. Contudo, análises mais específicas, como a cromatografia líquida de alta eficiência (HPLC) e a cromatografia a gas (GC) conseguem identificar e quantificar compostos individuais pertencentes à várias classes de compostos fenólicos. (CIULU et al., 2016; LORRAIN et al., 2013).

A cromatografia à gás, embora já tenha sido utilizada na análise de algumas classes de compostos fenólicos (SMEDS; EKLUND; WILLFÖR, 2016; VAIČIULYTE; BUTKIENE; LOŽIENE, 2016),ela não é a técnica mais empregada pois ainda existem dificuldade em analisar àqueles pertencentes à grupos que possuem baixa volatilidade e sensibilidade à altas temperaturas. (XU et al., 2017)

Dessa forma, devido a sua grande flexibilidade e sensibilidade, a HPLC é um dos métodos mais comuns para a análise de compostos fenólicos de matrizes de origem vegetal,

ela tem sido utilizada na separação de várias classes como antocianinas, taninos, flavanóis, flavonóis, ácidos fenólicos, entre outros (CAPRIOTTI et al., 2014).

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CAPÍTULO II

DETERMINATION OF CHLOROGENIC AND CAFFEIC ACIDS IN 64 FRUITS CONSUMED IN BRAZIL

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ABSTRACT

This study presents unpublished data on the content of chlorogenic acid and caffeic acid in fruits. Sixty-four fruits consumed in Brazil, most of which were produced in the country, were evaluated based on the levels of 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), 3,4-caffeoylquinic acid (3,4-DQA), 3,5-caffeoylquinic acid (3,5DQA) and 4,5-caffeoylquinic acid (4,5-DQA) and caffeic acid. The study investigated 15 fruits not yet reported in the literature in relation to these compounds, including several native species. The highest concentration of 3-CQA, 5-CQA and 4-CQA was observed in strawberry, cherry, blueberry, quince and blackberry; 3,4-DQA, 3,5-DQA and 4,5-DQA appeared with highest concentration in kumquat, passion fruit and sweet granadilla. Regarding caffeic acid, the highest content was found in blueberry and yellow pitaya. Considering the sum of compounds concentration, the fruits that stood out the most were quince, cherry, blueberry, blackberry and sweet granadilla, with concentrations between 200 $mg.kg^{-1}$ and 569.7 $mg.kg^{-1}$.

Keywords: phenolic compounds, mono-caffeoylquinic acid, dicaffeoylquinic acid, liquid chromatography, main components.

1. INTRODUCTION

The increase in global interest in relation to healthy habits and food stimulates the consumption of fruits, mainly because they are sources of vitamins, mineral salts and various bioactive compounds (DUTRA et al., 2017). Among these, the phenolic compounds are of importance due to the beneficial effects that they can have on health, related to their anti-inflammatory and antioxidant properties.

Phenolic compounds have a broad spectrum of structures, among which we can find chlorogenic acids, characterized as a quinic acid connected to trans-cinnamic acids (caffeic, ferulic or p-coumaric acids) through an ester bond (TAJIK et al., 2017). Chemically, they can be classified in relation to type, number and position of acyl residues (PARRAS et al., 2007). The biosynthesis pathway of chlorogenic acids begins by the deamination of L-phenylalanine to trans-cinnamic acid through the phenylalanine ammonia-lyase enzyme. Then, the trans-cinnamic acid, due to the action of cinnamate *4*-hydroxylase, forms the p-coumaric acid and, subsequently, the chlorogenic acids (AWAD et al., 2001). Hence, these compounds are normally available in vegetal food (NAVEED et al., 2018).

Studies have evidenced several benefits that chlorogenic acids can have on human health. According to Wan et al (2013), chlorogenic acids presented action of blood pressure reduction when they come from the extract of green coffee. The authors also noticed that chlorogenic acids significantly reduced both total cholesterol and LDL cholesterol, leading to increased HDL cholesterol. Huang et al. (2014) verified that chlorogenic acids suppressed the serum levels of lipids induced by a high-fat diet. Moreover, Ong, Hsu and Tan (2013) concluded that these polyphenols were able to inhibit the hepatic activity of glucose-6-phosphatase, decrease the hepatic steatosis and improve the lipid profile and absorption of skeletal muscle glucose, which in mice contributed to the improvement of fasting glucose levels, glucose tolerance, insulin sensitivity and dyslipidaemia. Additionally, they were linked to antitumour effects, with significant results against stomach cancer, colon cancer and even in the repression of related carcinogenic factors (MATSUNAGA et al., 2002b; SHAO et al., 2015). Studies performed by Yan et al. (2017) and Siswanto, Oguro and Imaoka (2017) showed that the chlorogenic acids were able to inhibit the development of liver carcinogenic cells. There was also verification of an inhibitory effect of chlorogenic acids on benign prostatic hyperplasia in mice, and their mechanisms may be related to the inhibition of the activity of 5-alpha reductase (HUANG et al., 2017b).

The main sources of chlorogenic acids already studied are coffee and tea, and *5*-CQA was reported as the most abundant isomer (MEINHART et al., 2017b; RODRIGUES; BRAGAGNOLO, 2013a). With regard to fruits, several studies reported the quantification of 5-CQA acid and caffeic acid in apple, pear, grape, kiwi, orange and guava (BATAGLION et al., 2015; CAN et al., 2014; FIORENTINO et al., 2009; HANAMURA; UCHIDA; AOKI, 2008; ÖZTÜRK et al., 2015; SANTOS et al., 2017; VÁZQUEZ-ARMENTA et al., 2017). These studies aimed to investigate phenolic acids and flavonoids without classifying and characterizing chlorogenic isomers as 3-CQA, 5-CQA, 4-CQA, 3,4-DQA, 3,5-DQA and 4,5-DQA. Pontes et al. (2002) studied 22 tropical fruits whose isomers were identified using a 5-CQA calibration curve. Other works, such as the one of Sánchez-Salcedo et al. (2016), only presented the identification of these compounds in fruits by mass spectrometry, without quantifying them.

Taking in account the diversity of fruits not fully investigated and data scarcity about the content of chlorogenic isomers, this study aimed at quantifying 3-CQA, 4-CQA, 5-CQA, 3,4-DQA, 3,5-DQA, 4,5-DQA acids and caffeic acid in 64 fruits consumed in Brazil, including several native species from the Amazon region and Brazilian *cerrado*, which have not yet been reported in the literature regarding the characterization of these compounds.

2. MATERIAL AND METHODS

2.1. SAMPLES AND REAGENTS

Sixty-four fruits were studied, and each one of them was obtained from three different suppliers (except when they were not available), totalling 171 samples (Table 1). The suppliers were from 16 Brazilian states, encompassing Southeast (São Paulo, Minas Gerais and Espírito Santo states), Northeast (Bahia, Paraíba, Ceará, Pernambuco, Sergipe and Rio Grande do Norte states), South (Paraná, Santa Catarina and Rio Grande do Sul states), North (Tocantins, Pará and Amazonas states) and Midwest (Goiás state). The samples were obtained at the maturation stage considered appropriate for raw consumption, and all of them were bought in Brazil, even though some were imported from other countries, such as the United States, Chile, Portugal, Spain, Colombia and Mexico. The sizes of fruits acquired was established according to two criteria: minimum of 0.5 kg for small fruits; and at least 3 kg for bigger ones (weighing between 0.8 kg and 8 kg).

The standards of caffeoylquinic acids (4-CQA, 5-CQA, 3,4-DQA, 3,5-DQA and 4,5-DQA) were obtained from Biopurify (Chengdu, China). As a commercial standard was not

found for 3-CQA acid, the identification of the compound in the samples was performed by comparing to the 3-CQA acid retention time and spectrum present in a sample from *yerba mate* (*Ilex paraguariensis)*, whose identification was confirmed by electrospray mass spectrometry in negative mode (Thermo, USA). The quantification of the 3-CQA acid was performed by employing the 5-CQA acid analytical curve. The stock solutions were prepared in HPLC grade methanol (J.T. Baker, Brazil), at 1 $mg.mL^{-1}$ concentration, and stored at -80°C. Formic acid was obtained from Merck (Brazil); HPLC grade acetonitrile was obtained from JT Baker (Brazil); and analytical grade ethanol, phenolphthalein and sodium hydroxide were obtained from Synth (Brazil). Water used in the experiments was ultra-purified in a Milli-Q® system (Millipore, USA), and all solutions were filtered in PVDF membranes of 0.22-µm porosity (Millipore, USA).

2.2. SAMPLES PREPARATION

Before the analyses, the samples were identified regarding the scientific and popular names according to data of EMBRAPA (Brazilian Agricultural Research Corporation) and photographed (Supplementary Material 1). The elaboration of the sample consisted in removing the non-edible parts and mashing the edible part (described in Table 1), employing cuts with knives and graters, followed by blending, crushing or processing, until the samples reached approximately 200 *mesh.* The samples were immediately analysed for verification of chlorogenic and caffeic acids and to determine moisture, total soluble solids (TSS) and total titratable acidity (TTA), the samples were stored at -18 °C up to the moment of being analysed.

2.3. ANALYSIS METHODS

Analyses of moisture, TSS and TTA (% in citric acid) were performed according to the methods described by the Association of Official Analytical Chemists (AOAC, 1995). For this purpose, a vacuum chamber (Tecnal, Brazil) and refractometer (Reichert Technologies, Germany) were used. The ratio was obtained through division of TSS values by TTA values.

For the analysis of chlorogenic and caffeic acids, 1 g of sample was weighed in a Falcon® 50 mL tube, to which 15 mL of a water:ethanol mixture (74:26) was added, according to the methodology described by Meinhart *et al.* (2017). The tube was hermetically sealed and shaken in a water bath at 60 °C at 240 rpm for 22 min. The sample was filtered through a paper filter, and the liquid extract was filtered through a PVDF membrane filter

with 0.22-µm porosity. Exceptionally, the extracts of avocado, alligator pear and coconut contained lipids, which were extracted by ether partitioning. One other exception was the açai sample, where the fruits were immersed in water, with a proportion of 60 ml of water to 40 g of açai, at 60 °C for 60 min before extraction, and then, the pulp was macerated and separated from seeds. The extract obtained with the macerated pulp was analysed considering the incorporated water content.

Chlorogenic and caffeic acids analyses were performed by high-performance liquid chromatography with a diode array detector (HPLC-DAD), using an Agilent Technologies equipment (Germany) model 1260, equipped with an automatic injector and quaternary pump, in Zorbax Eclipse Plus C18 column (Agilent Technologies, Germany), 4.6 mm ids, 100 mm long and 3.5 µm particle size, maintained at 30 °C, according to the method described by Meinhart *et al.* (2017), with adaptations.

The mobile phase was composed of acetonitrile (A) and water acidified with 0.1% formic acid, pH 2.4 (B). Elution was conducted by linear gradient system starting with 10% A, followed by 40% A at 6 min and 100% A at 6.1 min, maintained at 100% until 7.5 min for column cleaning. The column was reconditioned with 10% A for the next injection, from 7.6 min to 11 min. The mobile phase flow rate was of 1.2 $mL.min^{-1}$, with a 30-µL injection volume. The identification of compounds was performed by comparison with standards through retention time, absorption spectrum of diode array detection (DAD) at 325 nm, and co-chromatography. The quantification was through an external calibration curve of the analytical standards.

The method was validated according to recommendations of IUPAC (THOMPSON; ELLISON; WOOD, 2002). The limits of detection and quantification were established as concentration corresponding to signal of 3 and 6 times the signal-to-noise ratio, respectively. The value of 6 times the signal-to-noise ratio was established because at this concentration, the precision of the compounds presented a standard deviation lower than 10% with the employment of 7 successive determinations. A chromatogram with the respective retention times and elution order can be seen in Supplementary Material 2

The analytical curve was constructed with 6 equidistant points, in random triplicates, starting at the limit of quantification and ending at the concentration where the linearity was assured through the evaluation of the models regarding the lack of adjustment and significance of the regression according to ANOVA, performed through Statistica 7.0 software (Statsoft, USA). The precision was evaluated through successive determinations on the same day (n=10) and on different days (n=3), both at 3 levels, including the limit of

quantification, an intermediary point, and the maximum point of the analytical curve. The accuracy was evaluated through recovery testing in orange samples at the same levels of concentration as the accuracy, in triplicate.

The results of the analyses were submitted to exploratory analysis through principal component analysis (PCA), using Pirouette software (Infometrix, 2003). For performance of this analysis, the data were auto-scaled to present the same magnitude of response (mean equal to zero and standard deviation equal to one).

3. RESULTS AND DISCUSSION

3.1. MOISTURE, TOTAL SOLUBLE SOLIDS (TSS) AND TOTAL TITRATABLE ACIDITY (TTA)

Table 1 presents the results for moisture, TSS, TTA and ratio analyses. TSS and TTA measures are important in evaluations of fruits' post-harvest quality, and they are used to make inferences about their maturation level (NOOKARAJU et al., 2010). As previously mentioned, the analyses were performed on 171 samples, where 159 samples had a moisture level between 70% and 97%, and six samples showed levels between 40% and 70%. Tamarind and jatoba fruit samples were found to have exceptionally different moisture levels of 10% and 21%, respectively. Regarding the analysis of TSS, the values oscillated between 1.4ºBx and 51ºBx, and most of the samples (165) presented values lower than 20 ºBx. Tamarind and jatoba fruit were again exceptional, showing the highest values, with 40 ºBx and 46 ºBx means, respectively. For TTA, 157 samples presented mean values between 0.03% and 2.1%. Cupuaçu (2.8%), passion fruit (3.2%), Tahiti lime (6.5%), key lime (8.2%) and tamarind (12.2%) are among the samples with the highest levels of TTA. The highest ratios were found in fruits including coconut, abiu fruit, custard apple, persimmon, apricot, sapodilla fruit and sugar cane, varying between 130 and 550. Some fruits presented great variation among the different suppliers, particularly apricot, custard apple, sweet orange, Palestinian sweet lime and sapodilla fruit, which presented ratios with variations between 2 and 12 times. In general, the fruits presented moisture, TSS and TTA values according to the studies reported in the literature.

Table 1 Identification and characterization regarding moisture, total soluble solids (TSS), total titratable acidity (TTA) and ratio (TSS/TTA) of the fruits samples

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Abiu fruit *(*Pouteria caimito)	1	BRA-Southeast	Pulp	85.56	±	0.04	11.93	±	0.12	0.06	±	0.00	190.66	±	0.94
	2	BRA-Southeast		85.89	±	0.25	10.91	±	0.00	0.08	±	0.00	135.59	±	5.61
	3	BRA-Southeast		84.47	±	0.15	10.20	±	0.20	0.06	±	0.00	169.34	±	11.40
Acerola *(Malpighia emarginata DC.)*	1	BRA-North	Pulp, peel, seed	91.62	±	0.08	5.33	±	0.46	0.85	±	0.02	6.26	±	0.65
	2	BRA-North		90.88	±	0.71	4.73	±	0.12	0.83	±	0.02	5.71	±	0.21
	3	BRA-Northeast		92.40	±	0.09	12.16	±	0.12	1.72	±	0.01	7.07	±	0.07
Açai fruit *(Euterpe olearacea Mart)*	1	BRA-North	Pulp, peel	77.18	±	0.51	1.47	±	0.12	0.13	±	0.00	11.50	±	1.33
	*	*			*			*			*			*	
	*	*			*			*			*			*	
Ambarella *(Spondias dulcis Som)*	1	BRA-Southeast	Pulp	77.76	±	0.13	13.63	±	0.12	0.48	±	0.00	28.24	±	0.28
	2	BRA-Southeast		77.46	±	0.11	15.80	±	0.00	0.71	±	0.00	22.16	±	0.04
	3	BRA-Southeast		82.30	±	3.08	13.40	±	0.00	0.65	±	0.02	20.58	±	0.59
Apricot *(Mammea american)*	1	BRA-North	Pulp	86.85	±	0.08	9.46	±	0.00	0.42	±	0.05	22.50	±	2.66
	2	BRA-North		74.55	±	0.08	16.60	±	0.00	0.07	±	0.00	252.90	±	17.02
	*	*			*			*			*			*	

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Atemoya *(Annona cherimola Mill x Annona squamosa L)*	1	BRA-Southeast	Pulp	71.60	±	0.67	22.27	±	0.12	0.20	±	0.00	113.12	±	1.11
	2	BRA-Southeast		74.00	±	0.37	22.67	±	0.31	0.26	±	0.01	85.95	±	4.06
	3	BRA-Southeast		76.90	±	0.34	17.60	±	0.20	0.25	±	0.02	70.35	±	5.79
Alligator pear *(Persea americana)*	1	BRA-Southeast	Pulp	81.56	±	0.08	4.85	±	0.12	0.08	±	0.00	57.18	±	2.35
	2	BRA-Southeast		81.95	±	0.25	4.77	±	0.23	0.19	±	0.01	25.57	±	1.45
	3	BRA-Southeast		77.56	±	0.01	6.10	±	0.20	0.18	±	0.01	33.42	±	1.14
Avocado *(Persea americana* var. Hass and Fuerte*)*	1	BRA-Southeast	Pulp	71.30	±	0.44	6.80	±	0.20	0.19	±	0.02	35.46	±	2.68
	2	BRA-Southeast		68.90	±	0.58	6.88	±	0.31	0.15	±	0.00	46.25	±	2.11
	3	BRA-South		72.10	±	0.78	8.20	±	0.00	0.13	±	0.01	64.41	±	4.80
Banana *(Musa paradisiaca)*	1	BRA-Southeast	Pulp, seed	75.69	±	0.11	19.07	±	0.23	0.30	±	0.00	63.36	±	1.26
	2	BRA-Northeast		73.58	±	0.11	19.00	±	0.00	0.32	±	0.01	59.74	±	1.49
	3	BRA-South		73.25	±	0.07	21.27	±	0.12	0.24	±	0.01	90.37	±	5.15
Blackberry *(Morus nigra)*	1	BRA-Southeast	Pulp, peel, seed	90.99	±	0.44	5.67	±	0.12	1.61	±	0.03	3.53	±	0.13
	2	BRA-Southeast		85.42	±	0.62	8.38	±	0.00	1.25	±	0.03	6.73	±	0.19
	3	BRA-Southeast		87.71	±	0.38	5.13	±	0.12	1.44	±	0.03	3.58	±	0.12

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Blueberry (*Vaccinium myrtillus)*	1	USA	Pulp, peel, seed	82.91	±	0.15	11.60	±	0.00	0.58	±	0.04	20.20	±	1.24
	2	BRA-Southeast		88.65	±	0.01	8.53	±	0.12	1.38	±	0.02	6.19	±	0.11
	3	USA		84.35	±	0.06	12.20	±	0.00	0.47	±	0.00	25.84	±	0.12
Brazilian cherry *(Eugenia uniflora)*	1	BRA-Southeast	Pulp, peel	86.39	±	0.12	9.07	±	0.12	1.50	±	0.01	6.06	±	0.06
	*	*			*			*			*			*	
	*	*			*			*			*			*	
Cacao (*Theobroma cacao)*	1	BRA-Southeast	Pulp	80.47	±	0.50	15.73	±	0.12	0.44	±	0.01	36.15	±	0.40
	2	BRA-North		79.35	±	0.80	8.67	±	0.31	0.34	±	0.01	25.54	±	0.68
	3	BRA-Northeast		79.27	±	0.65	10.81	±	0.00	0.29	±	0.00	37.77	±	0.40
Cashew *(Anacardium occidentale)*	1	BRA-Northeast	Pulp, peel	89.22	±	0.70	8.80	±	0.00	0.24	±	0.00	35.93	±	0.69
	2	BRA-Northeast		88.90	±	0.49	6.87	±	0.12	0.20	±	0.00	34.09	±	0.29
	3	BRA-North		86.15	±	0.12	11.00	±	0.00	0.22	±	0.01	50.86	±	1.29
Cheese fruit (*Morinda citrifolia)*	1	BRA-South	Pulp, peel	86.73	±	0.08	8.20	±	0.00	0.55	±	0.03	15.02	±	0.70
	2	BRA-Northeast		89.67	±	0.16	6.65	±	0.00	0.10	±	0.01	64.71	±	6.65
	3	BRA-Southeast		88.58	±	0.19	6.45	±	0.00	0.49	±	0.02	13.21	±	0.41
Cherry *(Prunus avium)*	1	USA	Pulp, peel	76.48	±	0.04	20.94	±	0.00	0.78	±	0.01	26.69	±	0.41
	2	USA		83.84	±	0.04	15.30	±	0.12	0.63	±	0.01	24.27	±	0.37
	3	CHI		82.54	±	0.05	16.40	±	0.00	0.61	±	0.00	27.07	±	0.21

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Coconut *(Cocos nucifera)*	1	BRA-Northeast	Pulp	47.03	±	1.01	9.53	±	0.12	0.07	±	0.00	145.63	±	8.41
	2	BRA-Northeast		53.74	±	0.67	8.29	±	0.12	0.06	±	0.00	128.55	±	5.35
	3	BRA-Northeast		46.49	±	1.09	7.53	±	0.12	0.04	±	0.00	178.65	±	16.09
Common fig *(Fícus Carica L.)*	1	BRA-Southeast	Pulp, peel, seed	85.19	±	0.10	10.47	±	0.12	0.20	±	0.00	52.36	±	1.80
	2	BRA-Southeast		87.50	±	0.03	7.92	±	0.23	0.19	±	0.02	42.84	±	5.96
	3	BRA-Southeast		85.08	±	0.15	9.20	±	0.00	0.23	±	0.01	40.78	±	0.90
Common grape vine *(Vitis vinifera)*	1	CHI	Pulp, peel	77.03	±	0.08	18.18	±	0.12	0.43	±	0.02	42.53	±	1.39
	2	BRA-Northeast		80.06	±	0.11	17.15	±	0.00	0.32	±	0.02	53.57	±	3.80
	3	CHI		77.42	±	0.90	21.72	±	0.20	0.48	±	0.03	45.53	±	3.38
Common guava *(Psidium guajava)*	1	BRA-Southeast	Pulp, peel, seed	89.02	±	0.30	6.60	±	0.00	0.43	±	0.01	15.33	±	0.44
	2	BRA-Southeast		88.70	±	1.03	5.60	±	0.00	0.51	±	0.04	11.07	±	0.92
	3	BRA-Southeast		84.41	±	1.41	8.73	±	0.12	0.41	±	0.02	21.09	±	0.71
Common Plum *(Prunus domestica L)*	1	USA	Pulp, peel	84.07	±	0.01	12.61	±	0.12	1.62	±	0.01	7.81	±	0.09
	2	USA		84.63	±	0.15	10.91	±	0.12	1.46	±	0.08	7.50	±	0.42
	3	CHI		88.14	±	0.31	9.20	±	0.20	1.77	±	0.00	5.19	±	0.12
Cupucaçu *(Theobroma grandiflorum)*	1	BRA-North	Pulp	82.04	±	1.05	13.07	±	0.12	2.50	±	0.07	5.24	±	0.19
	2	BRA-Northeast		78.99	±	0.72	9.68	±	0.11	3.17	±	0.07	3.06	±	0.07
	*	*			*			*			*			*	

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Custard apple *(Annona squamosa)*	1	BRA-Northeast	Pulp	70.89	±	0.26	18.80	±	0.00	0.04	±	0.00	495.34	±	56.04
	2	BRA-Northeast		72.26	±	0.41	22.00	±	0.00	0.11	±	0.01	196.53	±	16.16
	3	BRA-Northeast		71.62	±	0.22	22.80	±	0.00	0.07	±	0.00	326.37	±	17.32
European pear *(Pyrus communis)*	1	POR	Pulp	84.31	±	0.08	12.57	±	0.00	0.09	±	0.00	135.45	±	6.42
	2	POR		82.65	±	0.29	14.20	±	0.00	0.11	±	0.00	129.86	±	4.71
	3	POR		87.43	±	0.30	9.80	±	0.00	0.08	±	0.00	130.07	±	0.93
Fuji apple *(Malus Communis)*	1	BRA-South	Pulp, peel	84.11	±	0.47	6.58	±	0.00	0.08	±	0.00	85.79	±	5.16
	2	BRA-South		84.73	±	0.13	11.04	±	0.12	0.18	±	0.02	62.75	±	5.00
	3	BRA-South		82.66	±	0.23	12.40	±	0.00	0.20	±	0.00	61.64	±	0.98
Genipap *(Genipa americana)*	1	BRA-North	Pulp	76.57	±	0.13	15.00	±	0.00	1.42	±	0.01	10.57	±	0.04
	2	BRA-North		75.35	±	1.09	14.40	±	0.00	1.47	±	0.00	9.79	±	0.01
	3	BRA-Northeast		83.04	±	4.29	12.19	±	0.12	1.16	±	0.02	10.51	±	0.23
Grapefruit (*Citrus paradisi)*	1	SPA	Pulp	92.67	±	0.05	7.30	±	0.00	1.47	±	0.01	4.96	±	0.05
	2	USA		90.07	±	0.21	9.67	±	0.06	1.85	±	0.04	5.24	±	0.15
	*	*			*			*			*			*	
Imbu (*Spondias tuberosa)*	1	BRA-South	Pulp, peel	88.21	±	0.20	6.80	±	0.00	2.10	±	0.03	3.24	±	0.05
	2	BRA-Southeast		86.78	±	0.12	10.20	±	0.00	1.53	±	0.03	6.66	±	0.12
	3	BRA-Northeast		84.35	±	0.41	9.53	±	0.12	1.32	±	0.13	7.27	±	0.74

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Jabuticaba *(Plinia cauliflora)*	1	BRA-Southeast	Pulp	82.51	±	1.08	12.33	±	0.12	1.31	±	0.00	9.40	±	0.08
	*	*			*			*			*			*	
	*	*			*			*			*			*	
Jackfruit *(Artocarpus integrifolia L.)*	1	BRA-Southeast	Pulp	76.14	±	0.45	17.80	±	0.00	0.32	±	0.01	55.15	±	1.13
	2	BRA-Southeast		85.03	±	0.34	5.73	±	0.12	0.48	±	0.01	11.84	±	0.44
	3	BRA-Northeast		69.82	±	0.09	23.87	±	0.12	0.51	±	0.01	47.13	±	0.85
Jamb *(Syzygium jambos)*	1	BRA-North	Pulp, peel	92.65	±	0.12	4.13	±	0.12	0.46	±	0.02	8.91	±	0.16
	2	BRA-North		90.44	±	0.10	5.67	±	0.12	0.59	±	0.03	9.61	±	0.65
	*	*			*			*			*			*	
Jatoba fruit *(Hymenaea courbaril)*	1	BRA-Northeast	Pulp	10.27	±	0.67	39.73	±	0.35	0.98	±	0.02	40.46	±	1.07
	2	BRA-Northeast		11.01	±	0.48	50.50	±	0.50	0.97	±	0.01	51.96	±	1.09
	3	BRA-North		9.55	±	0.18	47.76	±	0.29	1.03	±	0.06	46.67	±	2.57
Key lime *(Citrus aurantifolia)*	1	BRA-Southeast	Pulp	91.41	±	0.03	9.27	±	0.06	7.97	±	0.02	1.16	±	0.01
	2	BRA-Southeast		90.46	±	0.04	8.13	±	0.06	7.15	±	0.00	1.14	±	0.01
	3	BRA-Southeast		90.55	±	0.25	8.73	±	0.06	9.61	±	0.19	0.91	±	0.01

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Kiwi *(Actinidia deliciosa)*	1	BRA-South	Pulp, seed	84.32	±	0.16	12.07	±	0.12	1.57	±	0.03	7.67	±	0.19
	2	BRA-South		80.83	±	0.25	15.80	±	0.20	1.95	±	0.02	8.11	±	0.02
	3	BRA-South		84.64	±	0.25	12.13	±	0.20	1.46	±	0.00	8.33	±	0.15
Kumquat *(Fortunella)*	1	BRA-Southeast	Pulp, peel	82.08	±	0.27	13.00	±	0.31	0.21	±	0.01	62.91	±	1.44
	2	BRA-Southeast		81.81	±	0.05	14.77	±	0.31	0.27	±	0.01	54.40	±	2.95
	3	BRA-Southeast		83.94	±	0.15	11.93	±	0.31	0.19	±	0.00	63.33	±	1.50
Mango *(Mangifera indica)*	1	BRA-Northeast	Pulp	81.13	±	0.21	13.93	±	0.12	0.40	±	0.00	34.63	±	0.29
	2	BRA-Southeast		86.23	±	0.07	10.33	±	0.31	0.17	±	0.01	62.63	±	0.40
	3	BRA-Northeast		83.42	±	0.08	14.84	±	0.00	0.21	±	0.00	72.18	±	0.24
Mangosteen *(Garcinia mangostana)*	1	BRA-North	Pulp	84.02	±	0.23	12.39	±	0.12	0.39	±	0.01	32.08	±	0.75
	*	*			*			*			*			*	
	*	*			*			*			*			*	
Nectarine *(Prunus Persica)*	1	SPA	Pulp, peel	86.99	±	0.06	10.36	±	0.12	0.71	±	0.01	14.62	±	0.13
	2	SPA		88.63	±	0.02	9.62	±	0.11	0.71	±	0.03	13.51	±	0.68
	3	CHI		88.03	±	0.08	10.29	±	0.12	0.63	±	0.00	16.34	±	0.13
Palestinian sweet lime *(Citrus limettioides)*	1	BRA-Southeast	Pulp	93.34	±	0.45	9.07	±	0.06	0.04	±	0.00	227.83	±	11.90
	2	BRA-Southeast		92.49	±	1.85	7.20	±	0.00	0.04	±	0.00	173.10	±	2.42
	3	BRA-Southeast		92.61	±	0.10	7.47	±	0.06	0.06	±	0.00	117.56	±	2.07

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Papaya *(Carica papaya L)*	1	BRA-Northeast	Pulp	86.47	±	0.06	12.06	±	0.00	0.14	±	0.00	87.20	±	1.80
	2	BRA-Southeast		86.23	±	0.07	10.33	±	0.31	0.17	±	0.01	62.63	±	0.40
	3	BRA-Southeast		87.36	±	0.03	10.63	±	0.20	0.13	±	0.00	79.21	±	0.70
Passion fruit *(Passiflora edulis Sims)*	1	BRA-Southeast	Pulp, seed	80.17	±	0.31	10.47	±	0.12	2.34	±	0.02	4.48	±	0.06
	2	BRA-Southeast		83.47	±	0.02	19.20	±	1.04	3.64	±	0.06	5.27	±	0.22
	3	BRA-Southeast		82.99	±	0.69	12.16	±	0.12	3.73	±	0.16	3.27	±	0.17
Peach palm fruit (*Bactris gasipaes)*	1	BRA-North	Pulp	41.91	±	0.02	12.27	±	0.31	0.43	±	0.01	28.38	±	0.54
	*	*			*			*			*			*	
	*	*			*			*			*			*	
Peruvian groundcherry *(Physalis peruviana)*	1	BRA-South	Pulp, peel, seed	78.91	±	0.53	13.10	±	0.00	1.77	±	0.09	7.43	±	0.38
	2	COL		79.89	±	1.00	13.00	±	0.00	1.55	±	0.01	8.41	±	0.03
	3	COL		79.98	±	0.99	5.60	±	0.00	0.66	±	0.02	8.45	±	0.23
Persimmon *(Diosyrus kaki*)	1	BRA-Southeast	Pulp, peel	79.76	±	0.46	12.01	±	0.11	0.08	±	0.00	154.91	±	1.28
	2	BRA-Southeast		79.34	±	0.73	15.01	±	0.12	0.08	±	0.00	187.13	±	0.81
	3	BRA-Southeast		79.20	±	0.63	14.57	±	0.12	0.08	±	0.00	193.46	±	2.90

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Pineapple *(Ananas comosus L. Merril)*	1	BRA-North	Pulp	85.59	±	0.10	12.68	±	0.00	0.66	±	0.00	19.26	±	0.02
	2	BRA-Southeast		87.62	±	0.08	11.60	±	0.40	0.84	±	0.01	13.83	±	0.65
	3	BRA-Southeast		87.67	±	0.09	9.00	±	0.00	0.64	±	0.01	13.96	±	0.13
Pomegranate *(Punica granatum)*	1	BRA-Southeast	Pulp	89.48	±	0.64	17.60	±	0.00	1.65	±	0.08	10.67	±	0.50
	2	BRA-Southeast		85.53	±	0.03	12.93	±	0.12	0.92	±	0.05	14.15	±	0.65
	3	BRA-Southeast		87.47	±	0.35	15.60	±	0.10	0.54	±	0.01	28.99	±	0.56
Quince (*Cydonia oblonga)*	1	BRA-Southeast	Pulp, peel	81.99	±	0.38	12.10	±	0.12	0.35	±	0.01	34.13	±	0.46
	*	*			*			*			*			*	
	3	ARG		81.70	±	0.33	10.88	±	0.11	0.26	±	0.01	41.35	±	1.31
Rambutan *(Nephelium lappaceum)*	1	BRA-Southeast	Pulp	81.65	±	0.20	13.16	±	0.00	0.78	±	0.04	16.95	±	0.83
	2	BRA-South		82.45	±	0.31	16.07	±	0.12	1.08	±	0.01	14.86	±	0.25
	3	BRA-Southeast		85.15	±	0.47	10.40	±	0.00	0.36	±	0.01	28.77	±	0.70
Raspberry *(Rubus idaeus)*	1	BRA-Southeast	Pulp, peel, seed	83.62	±	0.22	10.80	±	0.00	1.63	±	0.01	6.61	±	0.04
	*	*			*			*			*			*	
	*	*			*			*			*			*	

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Sapodilla fruit *(Manilkara acharas)*	1	BRA-North	Pulp	75.98	±	0.11	17.17	±	0.00	0.07	±	0.00	246.35	±	4.48
	2	BRA-North		83.36	±	7.30	8.28	±	0.00	0.26	±	0.02	31.85	±	2.77
	3	BRA-Northeast		77.32	±	0.11	16.16	±	0.00	0.07	±	0.00	237.35	±	5.48
Soursop (*Annona muricata*)	1	BRA-Northeast	Pulp	85.69	±	0.02	9.58	±	0.00	0.70	±	0.01	13.77	±	0.19
	2	BRA-Northeast		86.91	±	0.63	8.82	±	0.00	0.52	±	0.00	16.95	±	0.15
	3	BRA-Northeast		85.51	±	0.19	10.80	±	0.20	0.55	±	0.07	19.95	±	2.03
Starfruit (*Averrhoa carambola*)	1	BRA-Southeast	Pulp, peel	89.51	±	0.23	9.29	±	0.11	0.17	±	0.00	54.71	±	0.55
	2	BRA-Southeast		91.70	±	0.15	7.40	±	0.20	0.13	±	0.01	57.91	±	0.81
	3	BRA-Southeast		90.19	±	0.06	7.61	±	0.00	0.15	±	0.01	50.03	±	2.39
Strawberry *(Fragaria X ananassa Duch.)*	1	BRA-Southeast	Pulp, peel, seed	90.27	±	0.42	7.21	±	0.11	0.93	±	0.00	7.74	±	0.13
	2	BRA-Southeast		90.08	±	0.16	7.13	±	0.58	1.07	±	0.03	6.67	±	0.39
	3	BRA-Southeast		90.13	±	0.53	8.71	±	0.00	1.04	±	0.01	8.35	±	0.08
Sugar cane *(Saccharum officinarum)*	1	BRA-Southeast	Pulp	82.38	±	0.07	17.47	±	0.06	0.07	±	0.00	267.79	±	14.43
	*	*			*			*			*			*	
	*	*			*			*			*			*	
Sugar time peach *(Prunus persica)*	1	SPA	Pulp, peel	89.70	±	0.03	8.60	±	0.00	0.40	±	0.01	21.68	±	0.27
	2	CHI		88.74	±	0.10	9.13	±	0.23	0.26	±	0.02	35.25	±	2.53
	3	SPA		88.74	±	0.08	8.90	±	0.12	0.21	±	0.01	43.36	±	1.12

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Sweet Granadilla *(Passiflora ligularis)*	1	COL	Pulp	73.45	±	0.61	12.80	±	0.00	0.43	±	0.01	29.97	±	0.41
	*	*			*			*			*			*	*
	3	COL		74.17	±	0.43	12.40	±	0.00	0.41	±	0.00	29.96	±	0.21
Sweet orange *(Citrus sinensis)*	1	BRA-Southeast	Pulp	89.68	±	0.05	9.67	±	0.06	0.65	±	0.02	14.91	±	0.37
	2	BRA-Southeast		91.46	±	0.08	8.20	±	0.00	0.06	±	0.00	135.42	±	3.21
	3	BRA-Southeast		93.07	±	0.08	8.17	±	0.06	0.53	±	0.01	15.49	±	0.23
Tahiti lime *(Citrus aurantifolia)*	1	BRA-Southeast	Pulp	91.36	±	1.21	7.90	±	0.00	6.73	±	0.05	1.17	±	0.01
	2	BRA-Southeast		91.14	±	0.15	7.57	±	0.06	6.33	±	0.01	1.20	±	0.01
	3	BRA-Southeast		90.82	±	0.23	7.80	±	0.00	6.69	±	0.09	1.17	±	0.01
Tamarind *(Tamarindus indica L.)*	1	BRA-Southeast	Pulp	23.49	±	0.16	40.00	±	0.20	13.10	±	0.11	3.05	±	0.01
	2	BRA-Northeast		18.53	±	1.19	46.82	±	0.30	12.79	±	0.47	3.66	±	0.11
	3	BRA-Northeast		22.67	±	0.33	31.93	±	0.23	10.39	±	0.02	3.07	±	0.03
Tangerine *(Citrus reticulata)*	1	BRA-Southeast	Pulp	88.59	±	0.06	9.72	±	0.12	0.45	±	0.01	21.78	±	0.73
	2	BRA-Southeast		87.00	±	0.08	11.54	±	0.11	0.47	±	0.00	24.72	±	0.32
	3	BRA-Southeast		88.28	±	0.10	10.55	±	0.11	0.38	±	0.01	27.83	±	0.37

Table 1 (cont.)

Popular name *(Scientific name)*	Supplier	Origin of the sample	Used parts	% Moisture			TSS (ºBx)			TTA (% in citric acid)			Ratio (TSS/TTA)		
				Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Tomato *(Lycopersicon esculentum)*	1	BRA-Southeast	Pulp, peel, seed	96.18	±	1.18	3.82	±	0.00	0.25	±	0.01	15.04	±	0.35
	2	BRA-Southeast		95.46	±	0.18	3.61	±	0.00	0.31	±	0.03	11.78	±	1.13
	3	BRA-Southeast		94.75	±	0.15	4.27	±	0.00	0.35	±	0.01	12.31	±	0.38
Watermelon *(Citrullus lanatus)*	1	BRA-Midwest	Pulp	90.24	±	0.10	9.50	±	0.10	0.13	±	0.00	75.60	±	1.25
	2	BRA-Midwest		89.76	±	1.30	10.73	±	0.06	0.09	±	0.00	121.67	±	5.87
	3	BRA-Northeast		89.59	±	0.20	10.30	±	0.00	0.10	±	0.00	107.17	±	0.34
Yellow melon *(Cucumis melo L.)*	1	BRA-Northeast	Pulp	90.34	±	0.10	9.09	±	0.11	0.12	±	0.01	73.94	±	2.75
	2	BRA-Northeast		95.15	±	0.14	4.53	±	0.31	0.06	±	0.00	70.30	±	9.65
	3	BRA-Northeast		90.93	±	0.05	8.27	±	0.12	0.12	±	0.00	69.99	±	2.11
Yellow pitaya *(Cereus undatus)*	1	MEX	Pulp, seed	80.83	±	0.15	17.87	±	0.12	0.14	±	0.01	124.15	±	6.29
	2	MEX		81.39	±	0.10	17.00	±	0.00	0.13	±	0.01	126.35	±	5.45
	*	*			*			*			*			*	

*Supplier not found; BRA: Brazil; USA: the United States of America; CHI: Chile; COL: Colombia; ARG: Argentina; SPA: Spain; POR: Portugal; MEX: Mexico; BRA-Southeast: samples from São Paulo, Minas Gerais and Espírito Santo states; BRA-Northeast: samples from Bahia, Paraíba, Ceará, Pernambuco, Sergipe and Rio Grande do Norte states; BRA-South: samples from Paraná, Santa Catarina and Rio Grande do Sul states, and BRA-North: samples from Tocantinas, Pará and Amazonas states; BRA-Midwest: samples from Goiás state.

3.2. VALIDATION OF THE ANALYTICAL METHOD

Table 2 presents the figures of merit of the analysis method for chlorogenic and caffeic acids, showing that the analytical curves presented adequate linearity, with F-values for the lack of adjustment lower than the F-critical value (3.11), with 95% confidence. The limits of quantification were 0.01 $mg.kg^{-1}$, the accuracy values were below 10% for all levels, and the recovery varied between 94% and 104%, showing excellent method accuracy. The parameters were in accord with the limits established by IUPAC (THOMPSON; ELLISON; WOOD, 2002), showing that the method is acceptable for conducting quantitative analyses with adequate analytical safety.

3.3. CHLOROGENIC AND CAFFEIC ACIDS

Table 3 presents the results for determination of chlorogenic and caffeic acids.

Herein, 3-CQA acid was found in 12 samples from five fruits, with contents varying between 0.47 $mg.kg^{-1}$ and 199.14 $mg.kg^{-1}$; the most exceptional quantities were found in abiu fruit, quince and cherry. Bastos et al. (2015) studied 3-CQA quantification in cherry, and they found it to contain 160.00 $mg.kg^{-1}$, a value close to the one verified in this study (135.50 $mg.kg^{1}$ to 199.10 $mg.kg^{1}$). The study of Pontes et al. (2002) on abiu fruit sample did not find 3-CQA acid, whereas in this study, a content between 0.80 $mg.kg^{-1}$ and 21.30 $mg.kg^{-1}$ was quantified, showing great variation between samples from different suppliers. Such variations may be related to the differences between cultivars, cultivation practices and soil and climatic conditions (RICKMAN; BRUHN; BARRET, 2007). The quince samples presented quantities between 102.4 $mg.kg^{-1}$ and 124.4 $mg.kg^{-1}$, values equivalent to those found by Stojanovic et al. (2017) but different from the ones found by Pontes et al. (2002), who did not detect the presence of the compound. For 44 out of the 64 fruits analysed in this study, no research on the quantification of 3-CQA acid in their parts was found in the literature.

On the other hand, 5-CQA acid was found in 55 samples from 25 fruits, with contents between 0.19 $mg.kg^{-1}$ and 522.33 $mg.kg^{-1}$. The highest concentrations were for tangerine, abiu fruit, jackfruit, nectarine, quince, blueberry and blackberry. The quantity found by Gungdogdu et al. (2011) in blackberry samples was 310.00 $mg.kg^{-1}$, lower than that found in this study (436.20 $mg.kg^{-1}$). Ancillotti et al. (2016) analysed blueberry, finding 1320.00 $mg.kg^{-1}$ on a dry basis (194.00 mg/kg^{-1} on a wet basis, considering 85% moisture), values close to the samples of this study, with exception to the sample from supplier 2 (194.00 mg/kg^{-1}). Daud et al. (2017) reported the presence of 5-CQA acid in jackfruit varieties, but no

quantification was done. In this study, the amount was found to be 0.40 mg.kg^{-1} and 34.20 mg.kg^{-1}, while Pontes et al. (2002) verified presence of 3.7 mg.kg^{-1}. The quantified content in tangerine ranged from 23.40 mg.kg^{-1} to 28.70 mg.kg^{-1}, and this fruit, along with 17 other samples, has not yet been reported in the literature to contain this compound. Moreover, 5-CQA acid was present in greater concentration than other mono-caffeoylquinic acids, corroborating the literature in relation to study on fruits, tea varieties and coffee varieties (PONTES et al., 2002; RODRIGUES; BRAGAGNOLO, 2013a).

Among the samples studied, only 19 have been already reported in the literature on the quantification of 4-CQA, which in this study was present in 22 fruits (57 samples). The highest quantities found were in pomegranate (4.04 mg.kg^{-1} to 10.20 mg.kg^{-1}), tamarind (6.26 mg.kg^{-1} to 13.39 mg.kg^{-1}), blackberry (29.87 mg.kg^{-1}) and strawberry (12.02 mg.kg^{-1} to 80.25 mg.kg^{-1}). Blackberry and pomegranate have not been previously studied for this compound. For tamarind and strawberry, in the studies of Pontes et al. (2002) and Spínola, Pinto and Castilho (2015), the authors did not detect the presence of the compound. Considering mono-caffeoylquinic acids, 4-CQA presented lower concentration than 5-CQA, but it was present in an equivalent number of samples.

Regarding dicaffeoylquinic acids, data were not found in the literature for 47 fruits among the samples studied. On the other hand, 3,4-DQA acid was found in 22 samples from ten fruits, with contents between 0.06 mg.kg^{-1} and 9.08 mg.kg^{-1}. Groundcherry, cashew, blueberry and grapefruit were the fruits with the highest contents of this compound, for which comparative data were not found in the literature.

Herein, 3,5-DQA acid was found in 26 samples from nine fruits, and it was the dicaffeoylquinic acid present in the largest number of samples, with contents between 0.25 mg.kg^{-1} and 72.54 mg.kg^{-1}. Key lime, passion fruit and kumquat samples presented the greatest amount of this compound. The literature does not include data for key lime and kumquat, while for passion fruit, there is only one study on the fruit juice, whose value was 13.8 mg per 100 mL juice (SPÍNOLA; PINTO; CASTILHO, 2015). The 4,5-DQA acid, in turn, was found in 17 samples from ten fruits, with contents between 0.08 mg.kg^{-1} and 535.18 mg.kg^{-1}. Grape, apricot, kumquat and sweet granadilla samples presented the greatest concentrations of this acid. Among them, only apricot has been already mentioned in a quantification study of Pontes et al. (2002), where detectable quantities were not found.

Table 2 Figures of merit of validation of the analytical method employed in analysis of chlorogenic and caffeic acids in fruits by HPLC-DAD

Parameters		Compounds					
		Caffeic Acid	5-CQA	4-CQA	3,4-DQA	3,5-DQA	4,5-DQA
Linear range of the analytical curve ($mg.L^{-1}$)		0.02 a 10.0	0.02 a 10.0	0.02 a 10.0	0.02 a 10.0	0.02 a 10.0	0.02 a 10.0
F-value for linear model adjustment(1)		0.082	0.070	0.221	0.126	0.081	0.096
Recovery in orange sample (% recovered (n=3))	Level 1	100.72	99.21	95.70	103.53	98.86	93.77
	Level 2	104.22	100.06	102.06	100.14	101.72	100.50
	Level 3	94.35	100.01	99.02	99.76	101.55	98.85
Precision oh the day (n=7) in fortified orange samples, in relative standard deviation (%)	Level 1	1.55	1.15	2.76	2.13	2.02	4.39
	Level 2	0.52	1.68	1.69	0.27	0.32	0.26
	Level 3	068	0.66	0.95	0.59	0.53	0.64
Precision between days (n=3) in fortified orange samples, in relative standard deviation (%)	Level 1	2.21	9.34	5.04	4.30	1.56	3.17
	Level 2	6.16	4.90	4.87	4.33	5.05	4.11
	Level 3	5.67	3.55	2.50	3.74	5.44	7.94
Limit of Quantification ($mg.kg^{-1}$)		0.01	0.01	0.01	0.01	0.01	0.01
Limit of Detection ($mg.kg^{-1}$)		0.005	0.005	0.005	0.005	0.005	0.005

(1): The model presents adjustment with adaptation when F-calculated value is smaller than F-critical value$_{4,14}$ (3.11, with 95% confidence). Level 1: LQ; Level 2: Intermediate concentration of the analytical curve linear range; Level 3: Maximum concentration of the analytical curve linear range; Compounds: 3-CQA: 3-Caffeoylquinic acid, 4-CQA: 4-Caffeoylquinic acid, 5-CQA: 5-Caffeoylquinic acid, 3,4-DQA: 3,4-Dicaffeoylquinic acid, 3,5-DQA: 3,5-Dicaffeoylquinic acid, 4,5-DQA: 4,5-Dicaffeoylquinic acid

Table 3 Quantification of caffeic acid and isomers of chlorogenic acids

Fruit	Supplier	Quantification of caffeic acid and chlorogenic acids (mg.kg^{-1} in wet basis)																					Sum
		Caffeic			3-CQA			5-CQA			4-CQA			3,4-DQA			3,5-DQA			4,5-DQA			
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Abiu fruit	1		Nd		21.32	±	3.21	20.63	±	2.30	1.69	±	0.21		nd			nd			nd		43.64
	2		Nd		1.87	±	0.13		nd		1.49	±	0.02		nd			nd			nd		3.36
	3		Nd		0.82	±	0.05	1.81	±	0.03	2.21	±	0.16		nd			nd			nd		4.84
Açai fruit	1	0.68	±	0.05		nd			nd			nd			nd			nd			nd		0.68
	*		*			*			*			*			*			*			*		*
	*		*			*			*			*			*			*			*		*
Apricot	1		Nd			nd			nd		3.49	±	0.16		nd			nq		2.00	±	0.44	5.49
	2		Nd			nd		1.39	±	0.15		nd			nd			nd			nd		1.39
	*		*			*			*			*			*			*			*		*
Blackberry	1		Nd			nd		436.22	±	10.70	29.87	±	2.10		nd			nd			nd		466.09
	2		Nd			nd			nd			nd			nd			nd			nd		nd
	3		Nd			nd			nd			nd			nd			nd			nd		nd
Blueberry	1	59.66	±	2.59		nd		227.05	±	14.80		nd		9.08	±	2.95		nd			nd		295.79
	2		Nd			nd		532.33	±	34.07		nd			nd			nd			nd		532.33
	3		Nd			nd		209.68	±	15.26		nd		2.63	±	0.14		nd			nd		212.31
Brazilian cherry	1		Nd			nd		4.36	±	0.07	1.16	±	0.11		nd			nd			nd		5.52
	*		*			*			*			*			*			*			*		*
	*		*			*			*			*			*			*			*		*
Cacao	1		Nd			nd			nd			nd			nd			nd		0.14	±	0.02	0.14
	2		Nd			nd			nd			nd			nd			nd		0.09	±	0.01	0.09
	3		Nd			nd			nd			nd			nd			nd		0.24	±	0.04	0.24
Cashew	1		Nd			nd			nd		0.22	±	0.00	1.14	±	0.12		nd			nd		1.36
	2		Nd			nd			nd		0.65	±	0.02	2.33	±	0.26		nd			nd		2.98
	3		Nd			nd			nd		1.79	±	0.09	1.94	±	0.08		nd			nd		3.73

Table 3 (cont.)

Fruit	Supplier	Quantification of caffeic acid, chlorogenic acids and rutin (mg.kg^{-1} in wet basis)																				Sum
		Caffeic			3-CQA			5-CQA			4-CQA			3,4-DQA			3,5-DQA			4,5-DQA			
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Cheese fruit	1		Nd			nd			nd		3.92	±	0.22		nd			nd			nd		3.92
	2		Nd			nd			nd		1.34	±	0.11		nd			nd			nd		1.34
	3		Nd			nd			nd		2.86	±	0.40		nd			nd			nd		2.86
Cherry	1	1.51	±	0.07	199.14	±	7.29	5.28	±	0.45	6.87	±	0.26		nd		2.03	±	0.14		nd		214.83
	2	1.99	±	0.10	135.54	±	10.42	1.22	±	0.03	3.03	±	0.34		nd		0.74	±	0.02		nd		142.52
	3	1.33	±	0.04	139.14	±	9.85	1.46	±	0.23	3.07	±	0.22		nd		0.84	±	0.08		nd		145.84
Common grape vine	1		Nd			nd			nd			nd			nd		0.28	±	0.03		nq		0.28
	2		Nd			nd			nd			nd			nd		1.63	±	0.15	0.95	±	0.14	2.58
	3		Nd			nd			nd			nd		0.31	±	0.06	5.39	±	0.26	1.35	±	0.22	7.05
Common guava	1		Nd			nd			nd			nd		0.15	±	0.03		nd			nd		0.15
	2		Nd			nd			nd			nd		0.06	±	0.00		nd			nd		0.06
	3		Nd			nd			nd			nd		0.87	±	0.09		nd					0.87
Fuji apple	1		Nd			nd		16.84	±	1.86		nd			nd			±			nd		16.84
	2		Nd			nd		16.21	±	1.10		nd			nd			±			nd		16.21
	3		Nd			nd		2.31	±	0.23		nd			nd			±			nd		2.31
Genipap	1	0.70	±	0.00		nd		1.85	±	0.12	3.86	±	0.14		nd			nd			nd		6.41
	2	0.37	±	0.00		nd		4.11	±	0.08	6.74	±	0.34		nd			nd			nd		11.22
	3	0.34	±	0.02		nd		0.42	±	0.02	0.70	±	0.06		nd			nd			nd		1.46
Grapefruit	1		Nd			nd			nd			nd		1.99	±	0.02		nd			nd		1.99
	2		Nd			nd			nd			nd		5.49	±	0.06		nd			nd		5.49
	*		*			*			*			*			*			*			*		*
Imbu	1		Nd			nd			nd		5.01	±	0.12		nd			nd			nd		5.01
	2		Nd			nd			nd		1.66	±	0.02		nd			nd			nd		1.66
	3		Nd			nd		1.21	±	0.25	1.81	±	0.02		nd			nd			nd		3.02

Table 3 (cont.)

Fruit	Supplier	Quantification of caffeic acid, chlorogenic acids and rutin (mg.kg^{-1} in wet basis)																				Sum
		Caffeic			3-CQA			5-CQA			4-CQA			3,4-DQA			3,5-DQA			4,5-DQA			
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Jackfruit	1		nd			nd			nd			nd			nd			nd			nd		nd
	2		nd			nd		34.24	±	4.29	1.44	±	0.04		nd		1.31	±	0.10	0.50	±	0.04	37.49
	3		nd			nd		0.38	±	0.07		nd			nd			nd			nd		0.38
Key lime	1		nd			nd			nd			nd			nd		5.58	±	0.13		nd		5.58
	2		nd			nd			nd			nd			nd		4.01	±	0.09		nd		4.01
	3		nd			nd			nd			nd			nd		1.90	±	0.08		nd		1.90
Kiwi	1		nd			nd			nd			nd			nd		0.28	±	0.04		nd		0.28
	2		nd			nd			nd			nd		0.74	±	0.09	0.25	±	0.02		nd		0.99
	3		nd			nd			nd			nd			nd			nq			nd		nd
Kumquat	1	1.65	±	0.30		nd			nd			nd			nd		33.81	±	4.35	6.38	±	0.84	41.84
	2	3.02	±	0.56		nd			nd			nd			nd		72.54	±	1.62	13.06	±	0.55	88.62
	3	2.51	±	0.26		nd			nd			nd			nd		44.85	±	7.43	10.09	±	1.52	57.45
Mango	1		nd		0.59	±	0.05		nd			nd			nd			nd			nd		0.59
	2		nd		1.09	±	0.04		nd			nd			nd			nd			nd		1.09
	3		nd		0.47	±	0.08		nd			nd			nd			nd			nd		0.47
Mangosteen	1		nd			nd		5.93	±	0.53		nd			nd			nd			nd		5.93
	*		*			*			*			*			*			*			*		*
	*		*			*			*			*			*			*			*		*
Nectarine	1		nd			nd		11.51	±	0.93	0.61	±	0.02		nd			nd		0.24	±	0.02	12.36
	2		nd			nd		5.46	±	0.23	0.06	±	0.01		nd			nd		0.08	±	0.00	5.60
	3		nd			nd		40.38	±	2.34	0.10	±	0.02		nd			nd		0.59	±	0.10	41.07
Palestinian sweet lime	1		nd			nd		1.26	±	0.06		nd			nd			nd			nd		1.26
	2		nd			nd		0.49	±	0.04		nd			nd			nd			nd		0.49
	3		nd			nd		1.45	±	0.05		nd			nd			nd			nd		1.45

Table 3 (cont.)

Fruit	Supplier	Quantification of caffeic acid, chlorogenic acids and rutin (mg.kg^{-1} in wet basis)																				Sum
		Caffeic			3-CQA			5-CQA			4-CQA			3,4-DQA			3,5-DQA			4,5-DQA			
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Passion fruit	1		nd			nd		1.04	±	0.04	0.12	±	0.02		nd		57.63	±	5.12		nd		58.79
	2		nd			nd		1.02	±	0.06	0.77	±	0.00		nd		62.35	±	4.75		nd		64.14
	3		nd			nd		1.27	±	0.04	1.53	±	0.04		nd		14.53	±	1.12		nd		17.33
Peruvian groundcherry	1		nd			nd			nd		3.80	±	0.25	2.10	±	0.11	2.13	±	0.35		nd		8.03
	2		nd			nd		1.39	±	0.09	2.66	±	0.18	2.10	±	0.24	4.26	±	0.60		nd		10.41
	3		nd			nd		1.96	±	0.07	0.38	±	0.01	0.50	±	0.05	2.22	±	0.20	0.40	±	0.03	5.46
Persimmon	1	0.53	±	0.04		nd			nd			nd			nd			nd			nd		0.53
	2		nq			nd			nd			nd		0.53	±	0.06		nd			nd		0.53
	3		nq			nd			nd			nd			nq			nd			nd		Nd
Pomegranate	1		nd			nd			nd		9.64	±	0.24		nd			nd			nd		9.64
	2		nd			nd			nd		10.20	±	1.54		nd			nd			nd		10.20
	3		nd			nd			nd		4.04	±	0.11		nd			nd			nd		4.04
Quince	1		nd		102.35	±	7.32	103.37	±	5.72	7.54	±	0.79		nd			nd			nd		213.26
	*		*			*			*			*			*			*			*		*
	3		nd		124.39	±	12.50	140.39	±	19.30	5.26	±	0.34		nd			nd			nd		270.04
Rambutan	1		nd			nd		0.19	±	0.04		nd			nd			nd			nd		0.19
	2		nd			nd			nd			nd			nd			nd			nd		Nd
	3		nd			nd		6.29	±	0.92		nd			nd			nd			nd		6.29
Sapodilla fruit	1		nd			nd		2.96	±	0.17		nd			nd			nd			nd		2.96
	2		nd			nd			nd		7.11	±	0.54		nd			nd			nd		7.11
	3		nd			nd		4.40	±	0.36		nd			nd			nd			nd		4.40
Starfruit	1		nd			nd			nd			nd			nd			nd			nd		Nd
	2		nd			nd			nd			nd			nd			nd			nd		Nd
	3		nd			nd			nd			nd			nd			nd		0.41	±	0.02	0.41

Table 3 (cont.)

Fruit	Supplier	Quantification of caffeic acid, chlorogenic acids and rutin (mg.kg^{-1} in wet basis)																				Sum
		Caffeic			3-CQA			5-CQA			4-CQA			3,4-DQA			3,5-DQA			4,5-DQA			
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Strawberry	1		nd			nd			nd		12.02	±	0.60	0.41	±	0.04	1.85	±	0.06		nd		14.28
	2		nd			nd			nd		21.69	±	2.06	0.55	±	0.05	3.94	±	0.32		nd		26.18
	3		nd			nd			nd		80.25	±	1.02	0.78	±	0.03	3.15	±	0.20		nd		84.18
Sugar cane	1		nd			nd		0.84	±	0.07	6.36	±	0.07		nd			nd			nd		7.20
	*		*			*			*			*			*			*			*		*
	*		*			*			*			*			*			*			*		*
Sugar time peach	1		nd			nd		4.03	±	0.58	0.54	±	0.01		nd			nd			nd		4.57
	2		nd			nd		2.64	±	0.04	0.08	±	0.00		nd			nd			nd		2.72
	3		nd			nd		13.85	±	1.14	0.17	±	0.02		nd			nd			nd		14.02
Sweet granadilla	1		nd			nd			nd			nd			nd			nd		35.92	±	1.46	35.92
	*		*			*			*			*			*			*			*		*
	3		nd			nd			nd			nd			nd			nd		535.18	±	23.91	535.18
Sweet Orange	1		nd			nd		2.23	±	0.08	1.49	±	0.04		nd			nd			nd		3.72
	2		nd			nd		4.25	±	0.08		nd			nd			nd			nd		4.25
	3		nd			nd		1.12	±	0.01		nd			nd			nd			nd		1.12
Tahiti lime	1		nd			nd			nd		1.08	±	0.05		nd			nd			nd		1.08
	2		nd			nd			nd		1.13	±	0.02		nd			nd			nd		1.13
	3		nd			nd			nd		1.05	±	0.05		nd			nd			nd		1.05
Tamarind	1	0.25	±	0.04		nd		2.13	±	0.13	13.39	±	1.11		nd			nd			nd		15.77
	2	0.23	±	0.01		nd		1.19	±	0.11	11.83	±	1.61		nd			nd			nd		13.25
	3	1.29	±	0.11		nd		0.34	±	0.05	6.26	±	0.63		nd			nd			nd		7.89
Tangerine	1		nd			nd		23.37	±	1.00		nd			nd			nd			nd		23.37
	2		nd			nd		24.71	±	1.46		nd			nd			nd			nd		24.71
	3		nd			nd		28.73	±	0.18		nd			nd			nd			nd		28.73

Table 3 (cont.)

Fruit	Supplier	Quantification of caffeic acid, chlorogenic acids and rutin (mg.kg^{-1} in wet basis)																					Sum
		Caffeic			3-CQA			5-CQA			4-CQA			3,4-DQA			3,5-DQA			4,5-DQA			
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Tomato	1	0.65	±	0.01	0.53	±	0.03	5.17	±	0.30	4.61	±	0.04	0.50	±	0.03	0.67	±	0.07		nd		12.13
	2	0.72	±	0.03		nd		4.93	±	0.18	2.88	±	0.08	0.69	±	0.07		nq			nq		9.22
	3	0.70	±	0.05		nd		6.40	±	0.23	3.51	±	0.08	0.38	±	0.02	0.60	±	0.06		nd		11.59
Watermelon	1		nd			nd			nd		2.71	±	0.08		nd			nd			nd		2.71
	2		nd			nd			nd		1.28	±	0.01		nd			nd			nd		1.28
	3		nd			nd			nd		4.23	±	0.08		nd			nd			nd		4.23
Yellow pitaya	1	19.21	±	1.75		nd			nd			nd			nd			nd		1.91	±	0.37	21.12
	2	1.83	±	0.12		±		3.15	±	0.39		nd			nd			nd			nd		4.98
	*		*			*			*			*			*			*			*		*

Fruits with no compound detected

LOD: 0.0052 mg.kg^{-1}

Acerola
Ambarella
Atemoya
Avocado
Avocado
Banana
Coconut
Common fig
Common Plum
Cupuaçu
Custard apple
European pear
Jabuticaba
Jamb
Jatoba fruit
Papaya
Pineapple
Peach palm fruit
Raspberry
Soursop
Yellow melon

Caffeic: caffeic acid; 3-CQA: 3-caffeoylquinic acid; 5-CQA: 5-caffeoylquinic acid; 4-CQA: 4-caffeoylquinic acid; 3,4-DQA: 3,4-dicaffeoylquinic acid; 3,5-DQA: 3,5-dicaffeoylquinic acid; 4,5-DQA: 4,5-dicaffeoylquinic acid; Mean: Obtained through analysis triplicate; Results expressed in wet basis; SD: Standard deviation; nd: not detected; nq: not quantified (below the LOQ - - 0,0104 mg.Kg^{1}); * supplier not found.

Caffeic acid was found in nine distinct fruits, totalling 20 samples, with contents between 0.23 $mg.kg^{-1}$ and 59.66 $mg.kg^{-1}$. The fruits that presented the greatest contents were cherry, kumquat, yellow pitaya and blueberry. For blueberry, Ancillotti et al. (2016) determined a concentration of caffeic acid of 0.47 $mg.kg^{1}$, whereas the value found in this study was between zero (not detected) and 59.7 $mg.kg^{1}$. For kumquat and yellow pitaya, there is no research available on the edible parts studied by this work.

Regarding the sum of chlorogenic and caffeic acids, among the 107 samples (43 fruits) that presented detectable quantities, kumquat, passion fruit, strawberry, cherry, blueberry, quince, blackberry and sweet granadilla showed the largest quantities, ranging from 57.45 to 535.18 $mg.kg^{-1}$. Considering only the sum of mono-caffeoylquinic acids (3-CQA, 5-CQA and 4-CQA), higher values were found in strawberry, cherry, blueberry, quince and blackberry. The sum of dicaffeoylquinic acids (3,4-DQA, 3,5-DQA and 4,5-DQA) was higher in kumquat, passion fruit and sweet granadilla samples.

Some samples presented considerable quantitative (abiu fruit, jackfruit, sweet granadilla, genipap, nectarine, Fuji apple and blackberry) and qualitative (apricot, groundcherry, pitaya, sapodilla fruit and imbu) variations among the different suppliers, which may be related to several factors (cultivation, climate and soil conditions, etc.) (RICKMAN; BRUHN; BARRET, 2007).

Among the 64 fruits studied by this work, 15 were not reported in the researched literature to date on any of the compounds, namely, atemoya, ambarella, custard apple, jatoba fruit, kumquat, Palestine sweet lime, key lime, Tahiti lime, watermelon, yellow melon, groundcherry, Brazilian cherry, yellow pitaya, peach palm fruit and tangerine. Moreover, 27 fruits presented data of only one or two quantified compounds, and caffeic acid and 5-CQA acid were the most reported, which is probably due to high cost of analytical standards, low commercial availability and low stability.

3.4. EXPLORATORY ANALYSIS BY PCA

A multivariate exploratory analysis was employed to analyse the fruit samples regarding the abundance of chlorogenic and caffeic acids. In this analysis, the data matrix was composed of 43 fruits (considering only the fruits that had concentration of some of the analytes) by 7 analytes (using the mean value of the concentration found between repetitions and suppliers). Thus, the principal component analysis gave a 62.97% explanation of the data variance using three main components (PC) (Figure 1).

PC1 differentiated the blueberry from the other samples for its high concentration of 5-CQA, 3,4-CQA and caffeic acids (Figure 1A and B). On the other hand, PC2 differentiated three groups, one containing strawberry, quince and cherry samples, whose composition is associated with the high concentration of 3-CQA and 4-CQA acids, in relation to the other samples. However, strawberry does not have 3-CQA acid, and it is only placed in this group due to the high concentration of 4-CQA acid (Table 3). A group of samples composed of passion fruit, kumquat and sweet granadilla was found in the inferior quadrant correlated to chlorogenic acids, 3,5-DQA and 4,5-DQA acids. Another group found in PC2 (Figure 1B) showed the grouping of many samples at the centre, which comprises the samples with smaller concentrations of analytes, among which we find starfruit, cacao, guava, persimmon and kiwi samples. In this grouping, the samples from pitaya, blackberry, tamarind and pomegranate tend to be different from the large agglomeration, but they are related to no specific compound, probably due to the great variation between the types of existent compounds.

In Figure 1C and D, the differentiation between kumquat and passion fruit samples from sweet granadilla can be observed in PC3, where the first two are separated due to high content of 3,5-DQA, and the last one by the presence of 4,5-DQA.

The exploratory analysis of this data set indicated that eight samples can be distinguished from others, namely: blueberry, strawberry, cherry, quince, sweet granadilla, passion fruit, kumquat and blackberry, due to the high concentration of chlorogenic and caffeic acids. No correlations were found between the concentration of the compounds and moisture, TSS, TTA and sample origin. However, it is worth mentioning that the fruits with the highest concentrations presented moisture between 70% and 90%, TSS between 7 and 18, TTA between 1% and 3.2%, and ratios between 4 and 60.

A new PCA was performed with the data matrix by removing the eight samples with the highest content of the compounds (Figure 1E and F) to enable a visualization of the discriminations between the other samples. In PC1, the yellow pitaya sample is different from the others by the higher concentration of 4,5-CQA acid and caffeic acid. In PC2, it is possible to observe the differentiation of the samples with intermediate quantities, such as key lime, groundcherry, grapefruit and yellow pitaya, associated with the presence of greater quantity of dicaffeoylquinic acids, which, in this analysis, differ from the samples previously agglomerated. The samples of abiu fruit, nectarine and tangerine, associated with the presence of greater quantity of mono-caffeoylquinic acids, are also different from the others.

Among the fruits analysed in this study, some stand out because of higher levels of chlorogenic and caffeic acids. In addition, they have the dietary advantages of a high-fibre content, high concentration of carotenoids, vitamins, anthocyanins, phytosterols, minerals, among others, and low carbohydrate and lipid contents (NAIR; AUGUSTINE, 2018; TOBARUELA et al., 2018), thus providing an excellent alternative to develop a diversified diet regarding the sources of essential micronutrients.

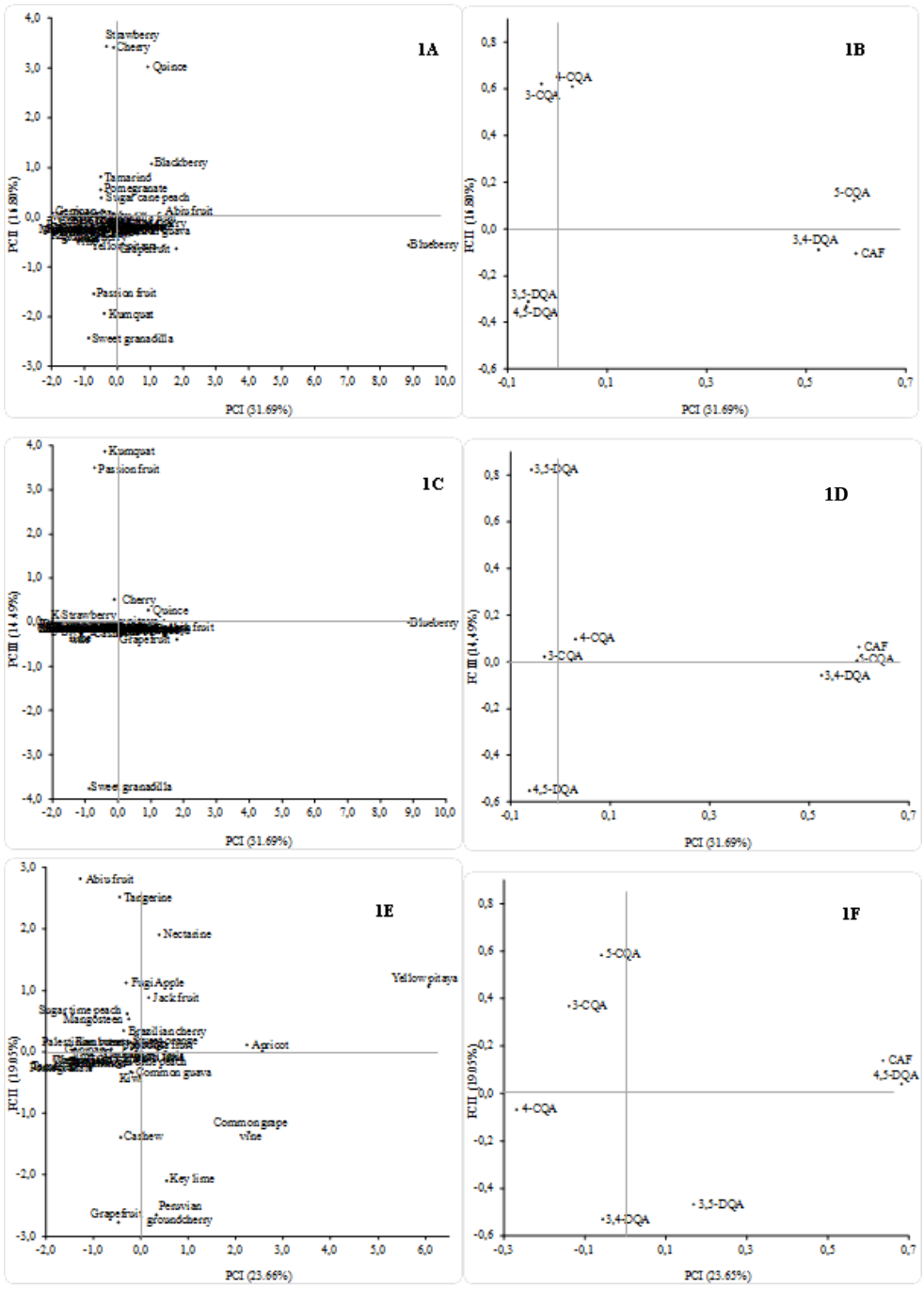

Figure 1 Principal componet analysis of samples of diferente fruits; Graph of Scores (1A, 1C) and loadings (1B, 1D) of combinations of the first 3 principal componentes. Figures 1E and 1F: PC1XPC2XPC3 of the averages chlorogenic and caffeic acid of fruits in which one or more compounds have been quantifies, without including the eight fruits with greater quantity (blueberry, strawberrym cherry quince sweet granadilla, passion fruit, kumquat and blackberry

4. CONCLUSION

This study evaluated the concentrations of seven compounds in a wide variety of fruits. Among the samples analysed, 67% presented quantifiable levels of one or more compounds. Blueberry and pitaya samples were the best sources of caffeic acid, and when considering the mono-caffeoylquinic acids (3-CQA, 5-CQA and 4-CQA), strawberry, cherry, quince and blackberry presented the highest values. Dicaffeoylquinic acids (3,4-DQA, 3,5-DQA and 4,5-DQA), in turn, were found in kumquat, passion fruit and sweet granadilla samples. This work gathered valuable information on 64 fruits, including 15 fruits for which the quantification of all chemical species investigated had not yet been reported. Some fruits have been shown to be important sources of chlorogenic and caffeic acids and may become the focus of future studies on technological processes and evaluations of in vivo effects.

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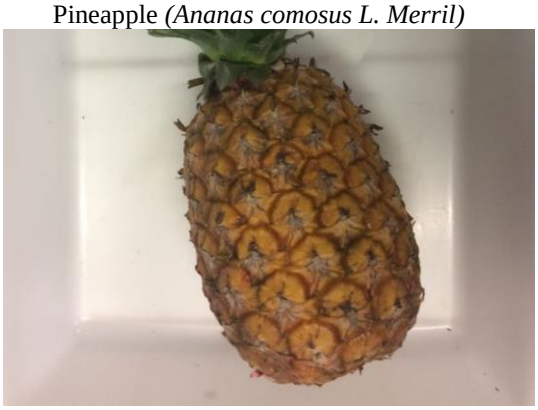
SUPPLEMENTARY MATERIAL 1

Avocado *(Persea americana)*

Açai fruit *(Euterpe olearacea Mart)*

Pineapple *(Ananas comosus L. Merril)*



Acerola *(Malpighia emarginata DC.)*

Abiu fruit (*Pouteria caimito)*

Common plum *(Prunus domestica L)*

Apricot *(Mammea american)*

Blackberry *(Morus nigra)*

Atemoya *(Annona cherimola Mill x Annona squamosa L)*

Ambarella *(Spondias dulcis Som)*

Avocado *(Persea americana* var. Hass e Fuerte*)*

Cashew *(Anacardium occidentale)*

Banana *(Musa paradisiaca)*

Sugar cane peach *(Saccharum officinarum)*

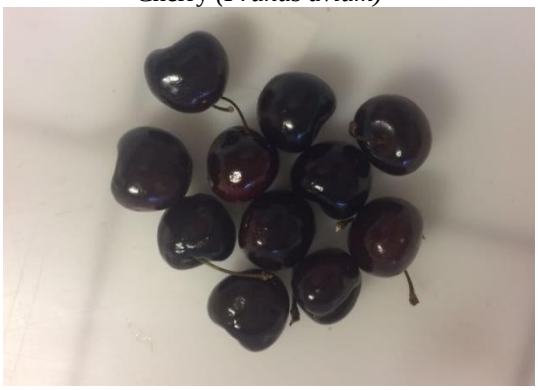
Cocoa (*Theobroma cacao)*

Persimmon *(Diosyrus kaki*)

Starfruit (*Averrhoa carambola*)

Common fig *(Fícus Carica L.)*

Cherry *(Prunus avium)*

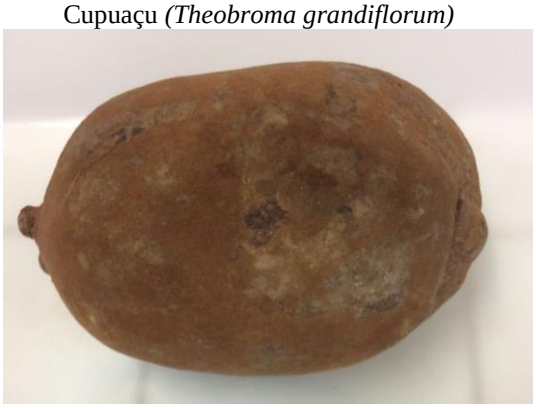


Raspberry *(Rubus idaeus)*

Coconut *(Cocos nucifera)*

Custard apple *(Annona squamosa)*

Cupuaçu *(Theobroma grandiflorum)*

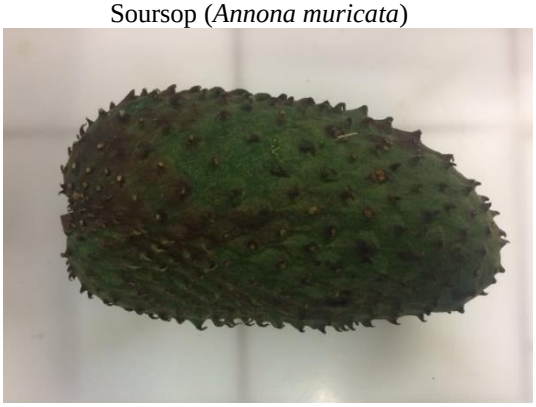


Genipap *(Genipa americana)*

Common guava *(Psidium guajava)*

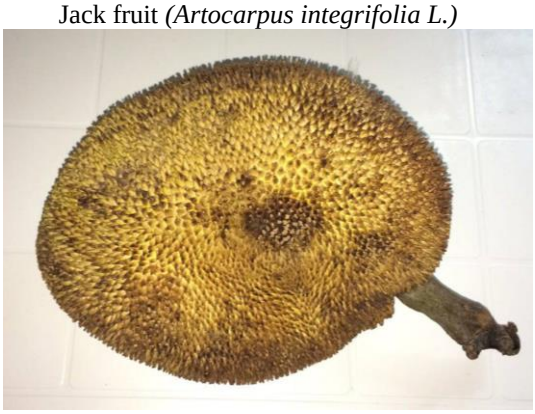
Sweet granadilla *(Passiflora ligularis)*

Soursop (*Annona muricata*)



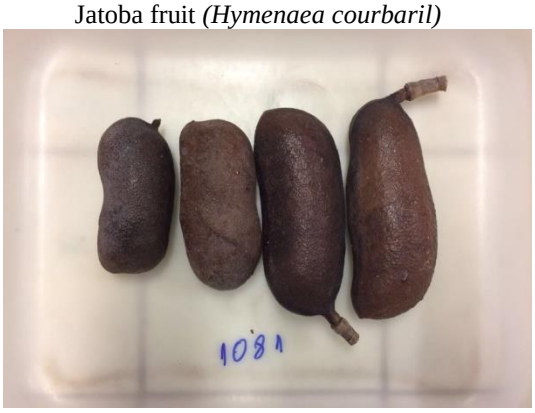
Jabuticaba *(Plinia cauliflora)*

Jack fruit *(Artocarpus integrifolia L.)*



Jamb *(Syzygium jambos)*

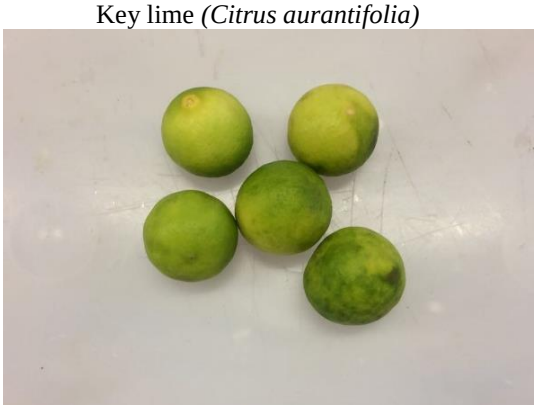
Jatoba fruit *(Hymenaea courbaril)*



Kumquat *(Fortunella)*

Kiwi *(Actinidia deliciosa)*

Key lime *(Citrus aurantifolia)*



Sweet orange *(Citrus sinensis)*

Fugi apple *(Malus Communis)*

Palestinian sweet lime *(Citrus limettioides*)

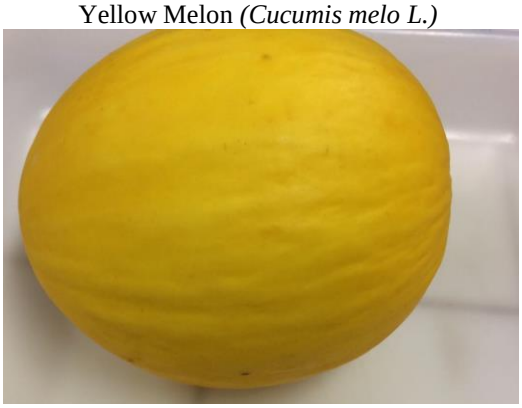
Papaya *(Carica papaya L)*

Tahiti lime *(Citrus aurantifolia)*

Mango *(Mangifera indica)*

Mangosteen *(Garcinia mangostana)*

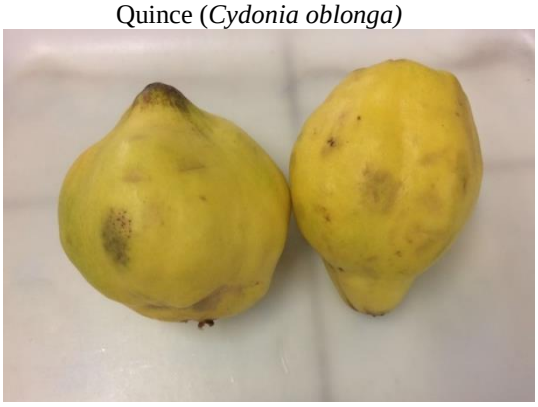
Yellow Melon *(Cucumis melo L.)*



Passion fruit *(Passiflora edulis Sims)*

Blueberry (*Vaccinium myrtillus)*

Quince (*Cydonia oblonga)*



Strawberry *(Fragaria X ananassa Duch.)*

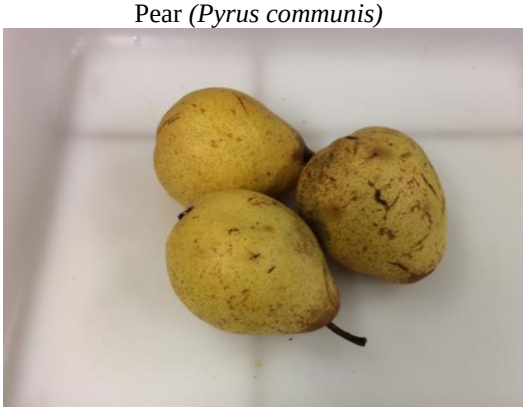
Watermelon *(Citrullus lanatus)*

Nectarine *(Prunus Persica)*

Cheese fruit (*Morinda citrifolia)*

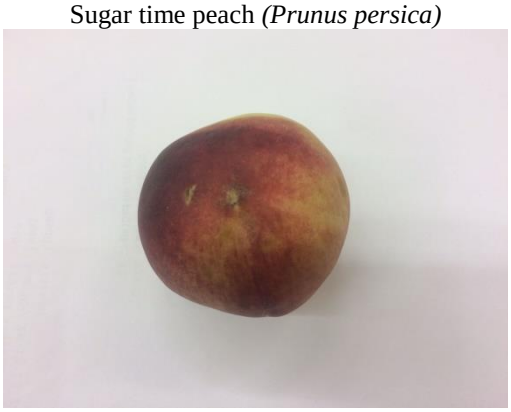
Physalis *(Physalis peruviana)*

Pear *(Pyrus communis)*

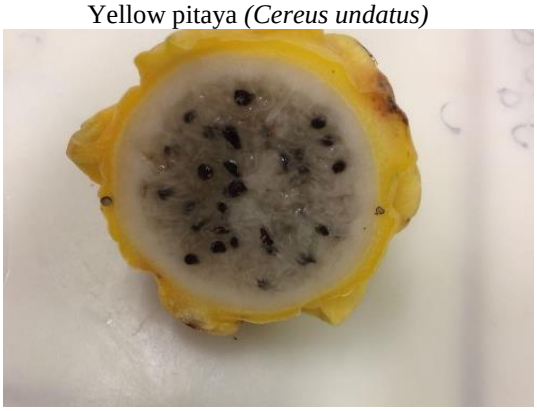


Brazilian cherry *(Eugenia uniflora)*

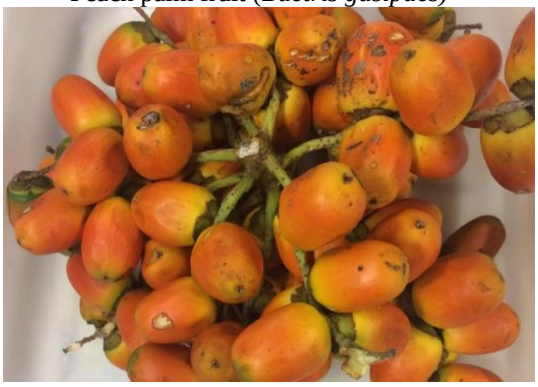
Sugar time peach *(Prunus persica)*



Yellow pitaya *(Cereus undatus)*



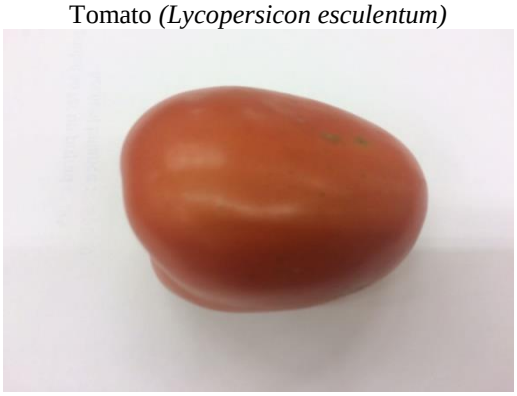
Peach palm fruit (*Bactris gasipaes)*



Rambutan *(Nephelium lappaceum)*

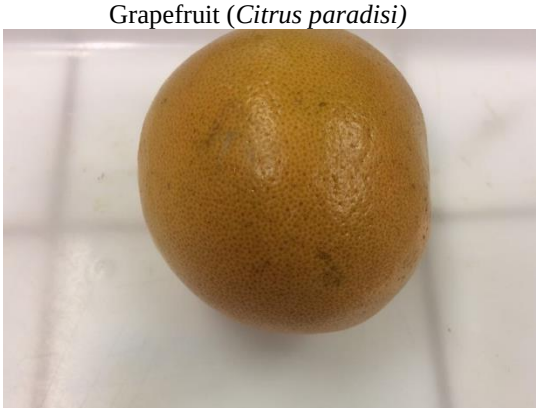
Pomegranate *(Punica granatum)*

Tomato *(Lycopersicon esculentum)*

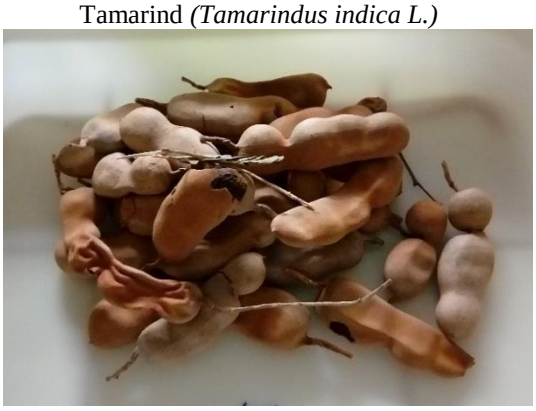


Sapodilla fruit *(Manilkara acharas)*

Grapefruit (*Citrus paradisi)*



Tamarind *(Tamarindus indica L.)*

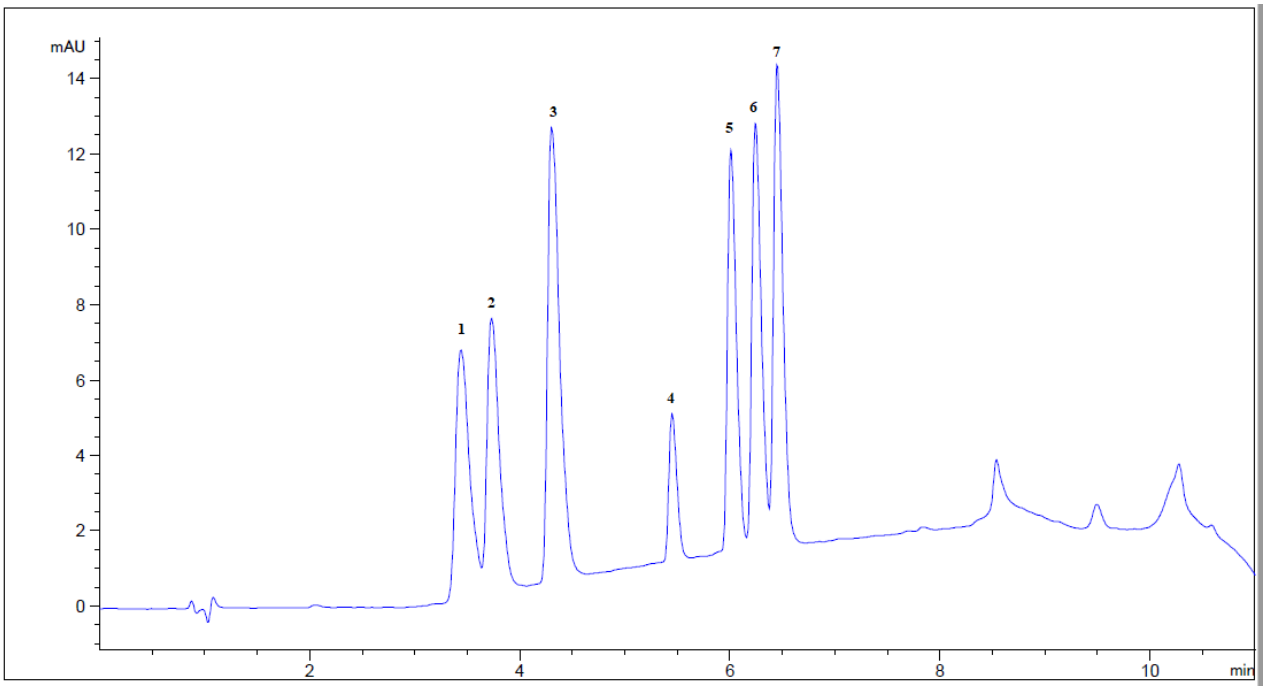


Imbu (*Spondias tuberosa)*

Tangerine *(Citrus reticulata)*

Common grape vine *(Vitis vinifera)*

SUPPLEMENTARY MATERIAL 2



1 – 5-CQA (3,47 min); 2 – 4-CQA (3,76 min); 3 – Caffeic Acid (4,30 min); 4 – Rutin (5,40 min); 5 – 3,4-DQA (6,01 min); 6 – 3,5-DQA (6,2 min), 7 – 4,5-DQA (6,4 min)

Standard's chromatogram

CAPÍTULO III

SCREENING FOR ISOMERS OF CHLOROGENIC ACIDS AND CAFFEIC ACID IN VEGETABLES COMMERCIALIZED IN BRAZIL

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Paper submitted to Journal of Agricultural and Food Chemistry

ABSTRACT

The amounts of six isomers of chlorogenic acids (i.e., 3-caffeoylquinic (3-CQA), 4-caffeoylquinic (4-CQA), 5-caffeoylquinic (5-CQA), 3,4-dicaffeoylquinic (3,4-DQA), 3,5-dicaffeoylquinic (3,5 DQA), 4,5-dicaffeoylquinic (4,5-DQA)) and caffeic acid were analysed in 53 vegetables consumed in Brazil via high-performance liquid chromatography. For the monocaffeoylquinic acids (3-CQA, 5-CQA and 4-CQA), higher levels were found in collard greens and common chicory, and 5-CQA was shown to be present in higher concentrations than the others and in a greater number of samples (55). The dicaffeoylquinic acid concentrations (3,4-DQA, 3,5-DQA and 4,5-DQA) were higher in samples of bay leaves and mustard. Caffeic acid was found in 22 of the studied samples, with higher levels in oregano, rosemary, sage, basil and cilantro. When all seven compounds were analysed, the samples that showed the highest concentrations were bay leaf, mustard, celery, rosemary, collard greens and common chicory. This study presents unpublished data about the presence and the content of isomers of chlorogenic acids and caffeic acid in vegetables.

Keywords: phenolic compounds, monocaffeoylquinic, dicaffeoylquinic, liquid chromatography, principal components.

1. INTRODUCTION

Vegetables have a nutritional importance that is related to the presence of bioactive compounds, which promote health-beneficial and relevant effects in healthy eating (SHASHIREKHA; MALLOKARJUNA; RAJARATHNAM, 2015). The consumption of vegetables, fruits and other horticultural products is recommended in global dietary guidelines. The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recommend a minimum intake of 400 g of fruits and vegetables per day (SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018; WHO; FAO, 2004). The use of vegetables such as bulbs, sprouts, rhizomes, tubers, fruits, leaf vegetables, flowers, leguminous plants, stems, roots and condiments in the diet is favoured by their higher availability and lower cost when compared to fruits (DENG et al., 2013), in addition to adding flavour and aroma to food.

Phenolic compounds are secondary metabolites of plants and have received increasing attention in recent years due to their bioactivity. Such compounds can be classified into flavonoids and non-flavonoids and comprise a wide variety of molecules (CROZIER; JAGANATH; CLIFFORD, 2009; DE PAULA et al., 2017; MATTILA; HELLSTROM, 2007). Chlorogenic acids are non-flavonoids that comprise the hydroxycinnamates, such as caffeic, ferulic and p-coumaric acids which, when combined with quinic acid, form a range of structures known as caffeoylquinic, feruloylquinic and coumaroylquinic acids (CROZIER; DEL RIO; CLIFFORD, 2010; CROZIER; JAGANATH; CLIFFORD, 2009; KREMR et al., 2016). Widely distributed in nature, the most common chlorogenic acid is 5-CQA. However, other structural isomers are present in vegetables, such as 3-CQA and 4-CQA. Dicaffeoylquinic acids, such as 3,4-DQA, 3,5-DQA and 4,5-DQA, can also be found, although there are few reports in the literature regarding these acids (CLIFFORD, 2000; WILLEMS et al., 2016).

Chlorogenic acids are found in food, and several studies consider tea and coffee as the main sources of these compounds (CLIFFORD, 2000; MEINHART et al., 2017a; RODRIGUES; BRAGAGNOLO, 2013b). However, quantifications of caffeic acid and 5-CQA have also been reported in some vegetables such as zucchini, eggplant, broccoli, onion, spinach, peas, green pepper, okra and cabbage (MATTILA; HELLSTROM, 2007; NEACSU et al., 2015; PEDRESCHI et al., 2011). Nevertheless, there is little research on 3-CQA and 4-CQA, which have only been reported in studies with sage, rosemary, oregano and eggplant (CHUN et al., 2005; MEINHART et al., 2017a; NIÑO-MEDINA et al., 2017). Research on

vegetables is even scarcer with regard to dicaffeoylquinic isomers, which have been reported in yams, common chicory, rosemary, sage and sweet potatoes (CHAMPAGNE et al., 2011; ESATBEYOGLU et al., 2016; MEINHART et al., 2017a; PAPETTI et al., 2017; ZHENG; CLIFFORD, 2008).

Studies of chlorogenic acids have indicated that these compounds exhibit various beneficial effects on health, such as exhibiting antioxidant (WONGSA; CHAIWARIT; ZAMALUDIEN, 2012), anti-inflammatory (BAO et al., 2018; DOS SANTOS et al., 2006), anti-HIV (MCDOUGALL et al., 1998), anti-diabetic (BAO et al., 2018) and anti-carcinogenic (FENG et al., 2005) properties. Elgndi et al., (2017) performed studies *in vitro* with vegetable extracts – used as a source of caffeic and chlorogenic acids – and verified the reduction of carcinogenic activity in human ovarian cells. Extracts of cilantro were tested *in vivo* and found to prevent the formation of atherosclerotic plaque, promote anti-inflammatory responses, exhibit antihypertensive and antiarrhythmic effects, and present antiviral and anti-carcinogenic properties. The authors found that these effects were correlated with the presence of caffeic acid, 5-CQA, quercetin and apigenin (BARROS et al., 2012; MIDDLETON; KANDASWAMI; THEOHARIDES, 2000). Bao et al. (2018) showed the action of chlorogenic acids in attenuating oxidative stress and inflammation in diabetic nephropathy and protecting against diabetic kidney diseases *in vitro* and *in vivo*. Thus, according to these results, it has been proposed that dietary intervention with caffeoylquinic acids, combined with medication, can slow the progression of disease in diabetic patients.

Considering the beneficial effects of chlorogenic and caffeic acids on health, the scarcity of reports about dicaffeoylquinic acids (3,4-DQA, 3,5-DQA, 4,5-DQA) and the diversity of vegetables and other horticultural products available, this study aimed to identify and quantify chlorogenic (3-CQA, 4-CQA, -5-CQA, 3,4-DQA, 3,5-DQA, 4,5-DQA) and caffeic acids of 53 vegetables sold in Brazil to determine possible new sources of these compounds.

2. MATERIAL AND METHODS

2.1. SAMPLES AND REAGENTS

The samples of 53 vegetables were acquired from three separate suppliers (except for caxi, bitter melon, mustard and sage, for which three suppliers were not located), with a total of 153 samples. The suppliers were from South, Southeast and Northeastern Brazil and some samples were imported from Argentina, Chile and Peru, as described in Table 1. The quantities of sample acquired followed two criteria: a minimum of 0.5 kg of sample and at

least three units of each. Thus, the masses of the samples ranged from 0.5 kg to small vegetables (e.g., purple garlic) and approximately 8 kg for larger vegetables (e.g., three Japanese pumpkin units). The samples have been photographed and identified with the scientific and the common name according to the data of the Brazilian Agricultural Research Corporation (EMBRAPA) (Table 1).

The standards of caffeoylquinic acids (4-CQA, 5-CQA, 3,4-DQA, 3,5-DQA and 4,5-DQA) were acquired from Biopurify (Chengdu, China). For 3-CQA, no commercial standard was found, so the identification of the compound was done by comparison of retention time and absorption spectrum with yerba mate (*Ilex paraguariensis*), which had the 3-CQA identity confirmed by mass spectrometry (electrospray ionisation in the negative mode) (Thermo, USA). The quantification of 3-CQA was performed by using the analytical curve of 5-CQA. Stock solutions were prepared in HPLC-grade methanol (J.T. Baker, Brazil) at a concentration of 1 $mg.mL^{-1}$ and stored at -80 °C. Formic acid was purchased from Merck (Brazil), chromatographic grade acetonitrile was purchased from J.T. Baker (Brazil), and analytical grade ethanol, phenolphthalein and sodium hydroxide were purchased from Synth (Brazil). The water used in the experiments was ultra-purified in a Milli-Q ® system (Millipore, USA). All solutions were filtered through 0.22-μm PVDF membranes (Millipore Corporation, France).

2.2. SAMPLES PREPARATION

For the preparation of the samples, the non-edible parts were initially removed. The edible fractions, described in Table 1, were crushed to approximately 200 *mesh* by cutting with knives and graters, followed by the use of a blender, grinder or food processor. Parts of the crushed samples were immediately analysed in relation to chlorogenic and caffeic acids and parts were stored in a freezer at -18°C until further analysis.

2.3. ANALYSIS METHODS

The analysis of moisture, TSS (°Bx) and TTA (% citric acid) were carried out using the methods described by the Association of Official Analytical Chemists (AOAC, 1995), using a vacuum oven from Tecnal (Brazil) and a digital refractometer from Reichert Technologies (Germany). The *ratio* was obtained as the ratio between the values of TSS and TTA.

For the analysis of chlorogenic and caffeic acids, 1 g of sample was weighed in a Falcon ® 50 mL tube and supplemented with 15 mL of water:ethanol (74:26), in accordance with the methodology described by Meinhart et al., (2017). The hermetically sealed tube was agitated in a water bath at 60 °C, with 240 rotations per minute for 22 minutes. Afterwards, it was filtered through filter paper and a PVDF membrane filter with porosity of 0.22 µm.

The extracts obtained were analysed by following the method described by Meinhart *et al.* (MEINHART et al., 2017a) in a high-performance liquid chromatograph (Agilent Technologies 1260, Germany) equipped with a detector diode arrangement, automatic gun, quaternary pump and column oven at 30°C. A C18 Zorbax Eclipse plus column (Agilent Technologies, Germany) 4.6 mm i.d., 100 mm long and 3.5 µm particle size was used. Elution was conducted with a linear gradient system starting with 10% acetonitrile (A) and 90% water acidified with 0.1% formic acid, pH 2.4, (B) up to 40% of A and 60% of B after 6 min. At 6.1 minutes, 100% of A was transferred for 1.5 min to clean the column, followed by 3.5 minutes of reconditioning with initial FM for the next injection. The mobile phase flow rate was 1.2 $mL.min^{-1}$, and the injection volume was 30 µL. The identification of compounds was performed via co-chromatography by comparing to standards' retention times, absorption spectra at 325 nm. Quantification was carried out using an external calibration curve of analytical standards.

This method has been validated in accordance with the recommendations of IUPAC (THOMPSON; ELLISON; WOOD, 2002) and ANVISA (ANVISA, 2017) regarding the parameters: detection and quantification limits (corresponding to a signal of 3 and 6 times the noise signal, respectively), linear track (built with 6 equidistant points, in random triplicates, starting at the limit of quantification and increasing to the concentration where linearity was ensured through the validation of models), precision (assessed through successive determinations on the same day (n = 7) and determinations between different days (n = 3), both at 3 levels, including the limit of quantification, a halfway point and the maximum point of the analytical curve) and accuracy (through a recovery test in the broccoli sample, at the same concentration levels as in the precision measurement, in triplicate). Analytical curve data were validated as the adjustment of models, regression significance and distribution of waste by ANOVA, using the *Statistica 7.0 software* (Statsoft, USA).

The results were submitted to exploratory analysis with principal component analysis (PCA), using the programme Pirouette (Infometrix, 2003). To carry out this analysis, data were auto-scaled to have the same magnitude of response (mean equal to zero and standard deviation equal to one).

3. RESULTS AND DISCUSSION

3.1. CHARACTERIZATION

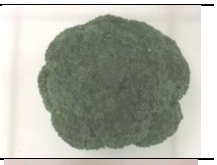
The characterization results of the samples regarding moisture, TSS, TTA and ratio are shown in Table 4. These measurements are important to facilitate comparisons with other studies. In the case of moisture, this was to relate our study to reports with results on a dry basis, and other measurements are employed to have a sense of the degree of vegetable maturation.

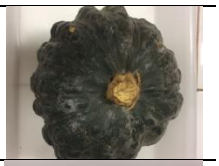
Of 153 samples, 131 showed a moisture degree greater than 80.0%, 17 samples between 61.1 and 78.0% and 5 samples between 37.8 and 55.9%. Bay leaf and cucumber samples had the lowest and the highest moisture content, with average values of 40.8% and 96.2%, respectively. For TSS, 146 samples had values between 1.2 and 9.6° Bx and 7 samples between 12.5 and 34.0° Bx. The highest TSS content was found in purple garlic and bay leaf, with average values of 33.4 and 13.8° Bx, respectively. The TTA ranged between 0.03 and 0.47; the highest average values in relation to the three suppliers were in the samples of purple garlic, bean sprouts and parsley, with averages of 0.35, 0.26 and 0.26%, respectively. The highest ratio was verified in samples of Japanese pumpkin, bay leaf and carrot (between 100.8 and 241.4). For the others, 33 samples had ratios between 54.4 and 97.4 and 114 samples between 9.9 and 48.3. The values found herein corroborate the results recorded in other studies (CANET, 2016; RASHIDI; GHOLAMI, 2011).

The variations between the suppliers for these results can be considered small for most of the 49 samples (obtained from three suppliers), showing relative standard deviation for moisture below 5.1%, except for samples of cassava, rosemary and bay leaf, which had values of 6.6, 8.9 and 9.1%, respectively. For TSS, 30 vegetables had variations lower than 20.0%, and 16 samples were between 21.9 and 36.7%. Three vegetables had the largest variation: chayote, arracacha and asparagus (42.9%, 43.8% and 70.1%, respectively). For acidity, 25 samples had relative standard deviations lower than 20.0%, and 16 samples were between 20.0 and 31.7%. Major variations occurred in the samples of celery, asparagus, cilantro, fresh peas, cassava, okra, basil and bean sprouts (between 44.0 and 70.8%). Variations in the ratio were between 1.3 and 19.6% in 24 samples, between 21.1 and 37.3% in 14 samples and between 41.9 and 67.3% in 11 samples, with the largest variations in bean sprouts and arracacha (54.3 and 67.3%, respectively).

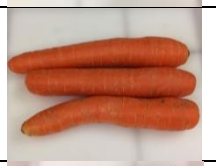
Table 1 Samples identification and characterization of moisture, TSS, TTA and ratio

Common Name (Scientific name)	Supplier	Sample source	Parties analyzed	Moisture (%)	TSS (° Bx)	TTA (% citric acid)	Ratio (TSS/TTA)	Photo
Sprouts								
Bean sprouts *(Vigna radiata)*	A	Bra-Southeast	Sprouts	95.1	3.0	0.2	15.5	
	B	Bra-Southeast	Sprouts	94.7	3.4	0.1	29.1	
	C	Bra-Southeast	Sprouts	93.1	4.6	0.5	9.9	
Bulbs								
Purple Garlic (*Allium sativum L.*)	A	ARG	Bulb	63.9	32.3	0.3	97.4	
	B	ARG	Bulb	63.5	34.0	0.4	90.7	
	C	ARG	Bulb	62.6	33.9	0.3	97.3	
Leek (*Allium ampeloprasum* L.)	A	Bra-Southeast	Bulb and leaves	92.6	4.1	0.1	54.1	
	B	Bra-Southeast	Bulb and leaves	93.2	3.0	0.1	27.5	
	C	Bra-Southeast	Bulb and leaves	90.7	3.5	0.1	25.8	
National Onion (*Allium cepa L.*)	A	Bra-South	Pulp	89.1	8.5	0.2	37.5	
	B	Bra-South	Pulp	92.5	4.4	0.2	24.8	
	C	Bra-Northeast	Pulp	90.4	6.5	0.2	29.1	
Condiments								
Rosemary (*Rosmarinus officinalis L.*)	B	Bra-Southeast	Leaves	70.8	4.3	0.1	36.7	
	D	Bra-Southeast	Leaves	72.3	5.9	0.2	34.8	
	E	Bra-Southeast	Leaves	61.1	5.3	0.2	35.4	
Chives (*Allium schoenoprasum L.*)	A	Bra-Southeast	Leaves and stalks	93.5	2.9	0.2	17.9	
	B	Bra-Southeast	Leaves and stalks	94.2	2.0	0.2	12.1	
	C	Bra-Southeast	Leaves and stalks	93.9	1.9	0.2	11.6	
Cilantro (*Coriandrum sativum L.*)	A	Bra-Southeast	Leaves and stalks	91.4	2.8	0.1	25.7	
	C	Bra-Southeast	Leaves and stalks	88.0	5.6	0.3	18.3	
	D	Bra-Southeast	Leaves and stalks	88.3	4.7	0.2	24.8	
Bay leaf (*Laurus nobilis L.*)	A	Bra-Southeast	Leaves	39.7	12.8	0.1	100.8	
	B	Bra-Southeast	Leaves	37.8	13.6	0.2	90.1	
	C	Bra-Southeast	Leaves	45.0	15.0	0.1	104.8	
Basil (*Ocimum basilicum L.*)	A	Bra-Southeast	Leaves and stalks	89.1	1.8	0.1	34.5	
	B	Bra-Southeast	Leaves and stalks	87.7	2.6	0.2	17.3	
	C	Bra-Southeast	Leaves and stalks	85.8	2.9	0.1	38.3	
Oregano (*Origanum vulgare*)	A	Bra-Southeast	Leaves	87.8	2.6	0.1	18.9	
	B	Bra-Southeast	Leaves	86.2	4.3	0.2	18.6	
	C	Bra-Southeast	Leaves	86.5	3.8	0.2	22.0	

Common Name (Scientific name)	Supplier	Sample source	Parties analyzed	Moisture (%)	TSS (° Bx)	TTA (% citric acid)	Ratio (TSS/TTA)	Photo
Parsley (*Petroselinum crispum (Mill.) Nym*)	A	Bra-Southeast	Leaves and stalks	86.2	6.1	0.3	19.7	
	B	Bra-Southeast	Leaves and stalks	89.9	3.9	0.2	19.1	
	C	Bra-Southeast	Leaves and stalks	88.3	5.7	0.3	20.4	
Sage (*Salvia officinalis*)	A	Bra-Southeast	Leaves	86.0	3.0	0.1	20.6	
	B	Bra-Southeast	Leaves	86.1	2.4	0.1	20.7	
Flowers								
Broccoli (*Brassica oleracea L. var. italica Plenck*)	A	Bra-Southeast	Leaves and stalks	90.5	3.8	0.2	18.9	
	B	Bra-Southeast	Leaves and stalks	90.7	4.3	0.3	16.6	
	C	Bra-Southeast	Leaves and stalks	88.4	4.5	0.2	18.0	
Cauliflower (*Brassica oleracea var. botritys*)	A	Bra-Southeast	Leaves and stalks	93.4	3.8	0.1	44.1	
	B	Bra-Southeast	Leaves and stalks	92.1	4.5	0.2	28.1	
	C	Bra-Southeast	Leaves and stalks	93.5	3.9	0.1	27.0	
Leaf vegetables								
Chard (*Beta vulgaris L. var. cicla*)	A	Bra-Southeast	Leaves and stalks	96.0	2.0	0.1	27.1	
	B	Bra-Southeast	Leaves and stalks	95.5	2.4	0.1	33.9	
	C	Bra-Southeast	Leaves and stalks	96.2	1.9	0.1	24.1	
Watercress (*Nasturtium officinale sp.*)	A	Bra-Southeast	Leaves and stalks	93.8	2.5	0.2	12.3	
	B	Bra-Southeast	Leaves and stalks	93.5	3.1	0.3	11.7	
	C	Bra-Southeast	Leaves and stalks	94.3	2.9	0.2	16.0	
Crisp lettuce (*Lactuca sativa L.*)	A	Bra-Southeast	Leaves and stalks	93.7	3.0	0.1	24.2	
	B	Bra-Southeast	Leaves and stalks	92.5	3.9	0.1	34.6	
	C	Bra-Southeast	Leaves and stalks	95.0	1.8	0.1	22.1	
Purple lettuce (*Lactuca sativa L.*)	A	Bra-Southeast	Leaves and stalks	94.5	2.4	0.1	26.7	
	B	Bra-Southeast	Leaves and stalks	94.6	3.1	0.1	26.7	
	C	Bra-Southeast	Leaves and stalks	94.6	2.8	0.1	25.6	
Common chicory (*Cichorium intybus L*)	A	Bra-Southeast	Leaves and stalks	92.2	2.4	0.1	28.6	
	B	Bra-Southeast	Leaves and stalks	93.5	1.6	0.1	16.2	
	C	Bra-Southeast	Leaves and stalks	91.9	2.3	0.1	21.7	
Chicory/Escarole (*Cichorium endivia L.*)	A	Bra-Southeast	Leaves	94.5	2.4	0.1	28.2	
	B	Bra-Southeast	Leaves	93.2	3.0	0.1	33.3	
	C	Bra-Southeast	Leaves	94.3	2.2	0.1	25.1	
Collard greens (*Brassica oleracea L. var. acephala D.C*)	A	Bra-Southeast	Leaves	90.4	5.8	0.3	22.2	
	B	Bra-Southeast	Leaves	91.0	4.3	0.2	21.1	
	C	Bra-Southeast	Leaves	91.2	4.3	0.2	21.4	

Common Name (Scientific name)	Supplier	Sample source	Parties analyzed	Moisture (%)	TSS (° Bx)	TTA (% citric acid)	Ratio (TSS/TTA)	Photo
Spinach (*Spinacea oleracea L.*)	A	Bra-Southeast	Leaves	94.0	2.2	0.1	33.8	
	C	Bra-Southeast	Leaves	93.6	2.2	0.1	26.3	
	D	Bra-Southeast	Leaves	93.1	3.9	0.1	41.9	
Mustard (*Brassica juncea (L.) Coss*)	A	Bra-Southeast	Leaves and stalks	92.7	3.1	0.2	19.6	
	B	Bra-Southeast	Leaves and stalks	95.3	3.5	0.2	17.5	
Cabbage (*Brassica oleracea L./ Brassica oleracea var. capitata ''f.alba''*)	A	Bra-Southeast	Leaves and stalks	93.6	3.2	0.1	53.9	
	B	Bra-Southeast	Leaves and stalks	93.6	4.3	0.1	48.3	
	C	Bra-Southeast	Leaves and stalks	93.8	4.0	0.1	42.5	
Purple Cabbage (*Brassica oleracea L./ Brassica oleracea var. capitata ''f.alba''*)	A	Bra-Southeast	Leaves and stalks	90.6	5.1	0.1	45.8	
	B	Bra-Southeast	Leaves and stalks	91.2	4.8	0.1	46.6	
	C	Bra-Southeast	Leaves and stalks	92.4	4.4	0.2	26.3	
Arugula (*Eruca sativa L.*)	D	Bra-Southeast	Leaves and stalks	93.8	2.8	0.2	16.8	
	A	Bra-Southeast	Leaves and stalks	94.0	3.2	0.1	24.1	
	B	Bra-Southeast	Leaves and stalks	92.9	3.2	0.1	23.0	
Fruits								
Japanese pumpkin (*Cucurbita moschata Duch (pumpkin) x Cucurbita máxima Duch (moranga)*)	A	Bra-Southeast	Pulp	84.6	9.1	0.0	241.4	
	B	Bra-Southeast	Pulp	87.4	6.9	0.0	156.3	
	C	Bra-Southeast	Pulp	90.0	5.8	0.0	177.1	
Italian Zucchini (*Cucurbita pepo L.*)	A	Bra-Southeast	Pulp, peel and seed	95.3	2.6	0.1	35.9	
	B	Bra-Southeast	Pulp, peel and seed	95.1	2.5	0.1	27.4	
	C	Bra-Southeast	Pulp, peel and seed	95.5	2.7	0.1	41.0	
Eggplant (*Solanum melongena L.*)	A	Bra-Southeast	Pulp, peel and seed	92.5	4.3	0.2	23.4	
	B	Bra-Southeast	Pulp, peel and seed	92.9	4.0	0.1	32.2	
	C	Bra-Southeast	Pulp, peel and seed	93.9	3.6	0.1	25.3	
Caxi/edible Porongo (*Cucurbita* sp.)	A	Bra-Southeast	Pulp and seed	90.0	4.4	0.1	53.6	
Chayote (*Sechium edule Sw*)	A	Bra-Southeast	Pulp	94.7	2.7	0.0	82.4	
	B	Bra-Southeast	Pulp	94.6	1.2	0.0	31.1	
	C	Bra-Southeast	Pulp	93.7	1.6	0.0	52.8	
Scarlet Eggplant (*Solanum gilo Raddi*)	B	Bra-Southeast	Pulp, peel and seed	92.0	3.9	0.1	29.5	
	D	Bra-Southeast	Pulp, peel and seed	90.1	5.2	0.2	26.8	
	E	Bra-Southeast	Pulp, peel and seed	90.0	4.3	0.1	34.2	

Common Name (Scientific name)	Supplier	Sample source	Parties analyzed	Moisture (%)	TSS (° Bx)	TTA (% citric acid)	Ratio (TSS/TTA)	Photo
Cackrey (*Cucumis anguria L*)	A	Bra-Southeast	Pulp and Peel	93.3	4.5	0.1	41.3	
	B	Bra-Southeast	Pulp and Peel	93.2	4.5	0.2	27.7	
	C	Bra-Southeast	Pulp and Peel	94.2	3.6	0.2	23.2	
Bitter melon (*Mormodica charantia L.*)	A	Bra-Southeast	Pulp and seed	93.2	2.9	0.2	19.0	
Japanese cucumber (*Cucumis sativus L.*)	A	Bra-Southeast	Pulp and seed	96.5	2.6	0.1	23.2	
	B	Bra-Southeast	Pulp and seed	95.6	2.7	0.1	21.8	
	C	Bra-Southeast	Pulp and seed	96.6	2.2	0.1	15.0	
Green pepper (*Capsicum annuum L.*)	A	Bra-Southeast	Pulp and Peel	94.1	5.7	0.2	34.2	
	B	Bra-Southeast	Pulp and Peel	93.9	4.3	0.1	32.3	
	C	Bra-Southeast	Pulp and Peel	93.5	4.5	0.1	33.0	
Okra (*Abelmoschus esculentus (L.) Moench*)	A	Bra-Southeast	Pulp, peel and seed	90.1	4.0	0.1	61.8	
	B	Bra-Southeast	Pulp, peel and seed	90.0	2.7	0.0	59.2	
	C	Bra-Southeast	Pulp, peel and seed	90.1	2.5	0.1	20.0	
Stems								
Asparagus (*Asparagus officinalis L.*)	A	PER	Leaves and stalks	93.7	12.5	0.2	60.6	
	B	CHI	Leaves and stalks	93.7	4.1	0.1	55.5	
	C	CHI	Leaves and stalks	93.6	4.1	0.1	28.2	
Celery (*Apium graveolens*)	A	Bra-Southeast	Leaves	93.3	2.6	0.1	26.8	
	B	Bra-Southeast	Leaves	92.2	3.7	0.1	25.1	
	C	Bra-Southeast	Leaves	88.2	4.6	0.2	19.3	
Leguminous plants								
Fresh Pea (*Pisum sativum L.*)	A	Bra-Southeast	Seed	75.1	9.6	0.1	75.7	
	B	Bra-South	Seed	76.4	9.3	0.1	95.0	
	C	Bra-South	Seed	77.6	8.3	0.2	33.4	
Green bean (*Phaseolus vulgaris L.*)	A	Bra-Southeast	Pod and seed	91.0	4.1	0.2	22.4	
	B	Bra-Southeast	Pod and seed	89.6	5.1	0.1	38.0	
	C	Bra-South	Pod and seed	91.7	3.2	0.1	30.7	
Lablab-bean[(*Lablab purpureus (L) Sweet*)	A	Bra-Southeast	Pod and seed	89.2	6.9	0.2	34.1	
	B	Bra-Southeast	Pod and seed	86.7	6.2	0.2	31.3	
	C	Bra-South	Pod and seed	88.1	5.5	0.2	26.4	
Roots								
Pink sweet potato (*Ipomoea potatoes L.*)	D	Bra-Southeast	Root	77.9	4.6	0.1	73.1	
	E	Bra-Southeast	Root	72.3	5.6	0.1	59.9	
	F	Bra-Southeast	Root	75.5	5.8	0.1	84.0	

Common Name (Scientific name)	Supplier	Sample source	Parties analyzed	Moisture (%)	TSS (° Bx)	TTA (% citric acid)	Ratio (TSS/TTA)	Photo
Beets (*Beta vulgaris L.*)	A	Bra-Southeast	Root	91.6	6.6	0.1	93.9	
	B	Bra-Southeast	Root	91.5	3.4	0.1	45.5	
	C	Bra-Southeast	Root	91.0	4.7	0.1	43.5	
Carrot (*Daucus carota L.*)	A	Bra-Southeast	Root	90.7	5.9	0.1	60.2	
	B	Bra-Southeast	Root	89.1	8.5	0.1	124.2	
	C	Bra-Southeast	Root	87.8	5.1	0.1	51.5	
Cassava (*Manihot esculenta Crantz*)	A	Bra-Southeast	Root	55.9	4.1	0.0	92.4	
	B	Bra-Southeast	Root	62.1	5.0	0.1	40.5	
	C	Bra-Southeast	Root	55.1	4.1	0.1	57.0	
Arracacha (*Arracacia xanthorrhiza Banc.*)	A	Bra-Southeast	Root	82.1	2.4	0.1	23.2	
	B	Bra-Southeast	Root	82.6	5.1	0.1	78.6	
	C	Bra-Southeast	Root	85.6	2.7	0.1	31.5	
Turnip (*Brassica rapa var. rapa (L.) Thell.*)	A	Bra-Southeast	Root and Peel	93.6	3.7	0.1	63.4	
	B	Bra-Southeast	Root and Peel	94.6	3.6	0.1	62.1	
	C	Bra-Southeast	Root and Peel	93.1	3.9	0.1	61.9	
Radish (*Raphanus sativus L.*)	A	Bra-Southeast	Root, peel and seed	95.6	2.6	0.1	26.5	
	B	Bra-Southeast	Root, peel and seed	95.7	2.1	0.1	30.0	
	C	Bra-Southeast	Root, peel and seed	95.2	2.8	0.1	55.1	
Rhizomes								
Ginger (*Zingiber officinale Roscoe*)	A	Bra-Southeast	Rhizome	84.5	4.2	0.1	55.5	
	B	Bra-Southeast	Rhizome	87.5	4.1	0.1	65.0	
	C	Bra-Southeast	Rhizome	87.7	2.7	0.1	39.2	
Purple Yam (*Colocasia esculenta L. Schott*)	A	Bra-Southeast	Rhizome	83.7	5.6	0.1	71.9	
	B	Bra-Southeast	Rhizome	85.0	4.2	0.1	33.4	
	C	Bra-Southeast	Rhizome	77.2	5.0	0.2	33.2	
Tubers								
English potato (*Solanum tuberosum ssp. Tuberosum*)	A	Bra-South	Tuber	84.8	3.1	0.2	17.5	
	B	Bra-South	Tuber	87.3	3.2	0.1	25.1	
	C	Bra-South	Tuber	83.6	2.8	0.1	21.3	
Yam (*Dioscorea alata L.; Dioscorea rotundata Poir; Dioscorea cayenensis*)	A	Bra-Southeast	Tuber	70.6	4.2	0.1	45.1	
	B	Bra-Southeast	Tuber	65.5	4.2	0.1	63.3	
	C	Bra-Southeast	Tuber	71.0	3.5	0.1	51.6	

3.2. VALIDATION

The validation parameters of the method of analysis by high-performance liquid chromatography showed results of 0.005 $mg.kg^{-1}$ for the detection limit and 0.01 $mg.kg^{-1}$ for the limit of quantification. The analytic curve proved to be linear for all compounds between 0.005 and 2.5 $mg.kg^{-1}$, with adjustment to the appropriate models (showing values of F between 0.07 and 0.2, below the critical F of 3.11, with 95% statistical reliability), significant models and random distribution of waste. In the limit of quantification, precision values obtained on each day (n = 7) were between 1.9 and 6.6% (expressed as relative standard deviation), in the intermediate concentration of the analytic curve (5.0 $mg.L^{-1}$) the precision was between 0.4 and 2.0%, and at high concentration between 1.3 and 2.7%. For the precision between days (n = 3) values of 0.1 and 7.1% were found, considering the three concentration levels. As to the accuracy of the method, the recovery levels varied for the three concentration levels, between 91.7 and 108.1%. The parameters are in accordance with the limits established by IUPAC (THOMPSON; ELLISON; WOOD, 2002) and by ANVISA (ANVISA, 2017), demonstrating that the method presented satisfactory analytical requirements for conducting quantitative analysis of samples.

3.3. DETERMINATION OF CHLOROGENIC ACIDS

3.3.1. Exploratory analysis

In Table 2, the results for the determination of chlorogenic and caffeic acids are presented.

Initially, exploratory multivariate analysis was used for the purpose of highlighting the vegetable samples regarding the abundance of chlorogenic and caffeic acids. In this analysis, the data matrix was composed of 39 vegetables (considering only those which had any concentration of analytes) and 7 analytes (using the mean value of the concentration found between repetitions and suppliers). Thus, the principal component analysis conducted on the data set showed that 60.0% of the variance could be explained by three principal components (PCs) (Figure 1).

PC1 discriminated the samples of bay leaf, rosemary, oregano and arugula from the other samples, due to the higher weights of 3,5-DQA and 4,5-DQA. In addition to these compounds, caffeic acid showed correlation with rosemary, oregano, sage and basil (Figure 1 A and B). On the other hand, in PC2, the celery and mustard samples are discriminated from the others because of high concentrations of 3,4-DQA and 5-CQA, while basil and asparagus

are also distinguished by containing higher concentrations of 4-CQA. In PC3, the high correlation of 3-CQA can be mainly observed with collard greens but also with the okra in relation to other samples, associating with the same high compound concentration (Figure 1C and D).

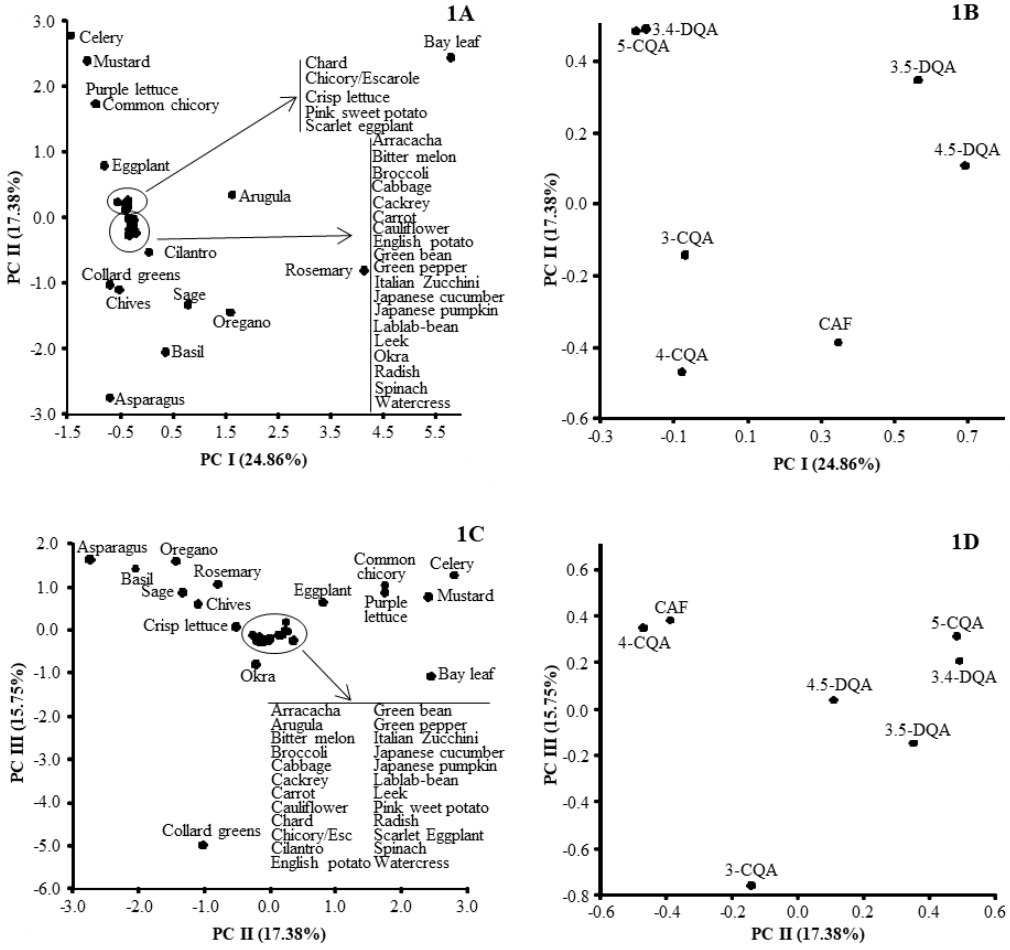


Figure 1 Scores (1A, 1C) and loadings (1B, 1D) plots showing the three principal components of the six isomers of chlorogenic acid: 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), 3,4-caffeoylquinic acid (3,4-DQA), 3,5-caffeoylquinic acid (3,5DQA) and 4,5-caffeoylquinic acid (4,5-DQA) and caffeic acid, in different samples of vegetables.

Table 2 Chlorogenic and caffeic acids composition in vegetables (mg.kg^{-1-} on wet basis)

Vegetable	Supplier	CAFFEIC	3-CQA	5-CQA	4-CQA	3.4-DQA	3.5-DQA	4.5-DQA	Sum
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Sprouts									
Bean sprouts	A	nd	nd	nd	nd	nd	nd	nd	0.0
	B	nd	nd	nd	nd	nd	nd	nd	0.0
	C	nd	nd	nd	nd	nd	nd	nd	0.0
Bulbs									
Purple Garlic	A	nd	nd	nd	nd	nd	nd	nd	0.0
	B	nd	nd	nd	nd	nd	nd	nd	0.0
	C	nd	nd	nd	nd	nd	nd	nd	0.0
Leeks	A	nd	nd	nd	nd	nd	3.3 ± 0.3	nd	3.6
	B	nd	nd	nd	nd	nd	1.8 ± 0.1	nd	1.7
	C	nd	nd	nd	nd	nd	3.2 ± 0.2	nd	3.2
National Onion	A	nd	nd	nd	nd	nd	nd	nd	0.0
	B	nd	nd	nd	nd	nd	nd	nd	0.0
	C	nd	nd	nd	nd	nd	nd	nd	0.0
Condiments									
Rosemary	B	26.8 ± 0.8	nd	nd	nd	nd	11.5 ± 0.3	44.2 ± 1.4	80.8
	D	45.5 ± 0.6	nd	nd	nd	nd	7.4 ± 0.5	62.9 ± 1.6	115.6
	E	54.3 ± 0.3	nd	nd	nd	nd	9.7 ± 0.2	49.9 ± 0.6	114.7
Chives	A	nd	nd	0.9 ± 0.1	12.0 ± 0.7	26.4 ± 4.6	nd	nd	36.6
	B	nd	nd	0.7 ± 0.1	14.6 ± 1.2	0.2 ± 0.0	nd	nd	16.9
	C	nd	nd	0.6 ± 0.1	14.5 ± 0.8	0.3 ± 0.0	nd	nd	14.5
Cilantro	A	13.8 ± 0.4	nd	nd	nd	nd	nd	nd	13.5
	C	15.1 ± 0.1	nd	nd	nd	nd	0.4 ± 0.0	nd	15.6
	D	10.0 ± 0.5	nd	nd	nd	nd	nd	nd	10.6

Vegetable	Supplier	CAFFEIC			3-CQA			5-CQA			4-CQA			3.4-DQA			3.5-DQA			4.5-DQA			Sum
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Bay leaf	A	nd			nd			nd			nd			nd			343.8	±	2.4	42.8	±	0.6	383.3
	B	nd			nd			nd			nd			nd			362.3	±	12.6	40.7	±	1.9	409.5
	C	nd			nd			nd			nd			nd			331.6	±	2.6	40.4	±	0.7	370.3
Basil	A	16.6	±	1.0	nd			1.4	±	0.0	12.4	±	1.2	nd			nd			nd			28.3
	B	39.1	±	1.9	nd			2.5	±	0.2	7.0	±	0.5	nd			nd			nd			51.2
	C	40.4	±	1.0	nd			2.2	±	0.0	18.9	±	0.2	nd			nd			nd			62.3
Oregano	A	27.8	±	1.8	nd			2.5	±	0.3	3.5	±	0.2	nd			0.6	±	0.0	2.3	±	0.0	39.0
	B	74.3	±	10.2	nd			9.8	±	0.3		±		nd			6.3	±	0.8	1.5	±	0.1	80.7
	C	64.4	±	2.7	nd			26.7	±	0.6	0.6	±	0.0	nd			1.8	±	0.3	23.7	±	1.0	118.1
Parsley	A	nd			nd			nd			nd			nd			nd			nd			0.0
	B	nd			nd			nd			nd			nd			nd			nd			0.0
	C	nd			nd			nd			nd			nd			nd			nd			0.0
Sage	A	22.3	±	0.6	nd			nd			nd			nd			nd			nd			23.0
	B	59.6	±	2.5	nd			nd			nd			nd			nd			nd			58.2
Flowers																							
Broccoli	A	nd			nd			2.3	±	0.3	2.4	±	0.2	nd			nd			nd			4.3
	B	nd			nd			1.7	±	0.1	1.2	±	0.0	nd			nd			nd			2.9
	C	nd			nd			2.8	±	0.4	3.1	±	0.4	nd			nd			nd			5.1
Cauliflower	A				nd						1.1	±	0.0	nd			nd			nd			1.1
	B	0.4	±	0.1	nd			4.8	±	0.6	1.6	±	0.2	nd			nd			nd			6,7
	C	0.1	±	0.0	nd			3.9	±	0.3	0.7	±	0.0	nd			nd			nd			4.2
Leaf vegetables																							
Chard	A	nd			nd			nd			2.9	±	0.1	49.4	±	3.1	nd			nd			54.5
	B	nd			nd			nd			4.3	±	0.4	51.2	±	7.2	nd			nd			64.1
	C	nd			nd			nd			2.5	±	0.0	31.3	±	3.1	nd			nd			30.3

Vegetable	Supplier	CAFFEIC			3-CQA			5-CQA			4-CQA			3.4-DQA			3.5-DQA			4.5-DQA			Sum
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Watercress	A	nd			nd			nd			nd			5.0	±	0.7	nd			nd			5.7
	B	nd			nd			nd			nd			9.1	±	0.4	nd			2.1	±	0.1	11.8
	C	nd			nd			nd			nd			8.8	±	0.4	nd			1.6	±	0.2	10.9
Crisp lettuce	A	nd			nd			23.5	±	2.2	0.5	±	0.0	nd			6.3	±	0.2	nd			31.5
	B	nd			nd			21.9	±	2.9	0.8	±	0.0	nd			1.3	±	0.0	nd			27.4
	C	nd			nd			2.6	±	0.3	0.1	±	0.0	nd			nd			nd			3.0
Purple lettuce	A	nd			nd			63.3	±	2.8	nd			nd			4.2	±	0.3	nd			70.9
	B	nd			nd			38.5	±	1.2	nd			nd			5.2	±	0.9	nd			42.4
	C	nd			nd			128.0	±	11.5	nd			nd			11.1	±	2.2	nd			129.2
Common chicory	A	nd			nd			53.7	±	2.3	1.6	±	0.0	nd			8.9	±	0.9	nd			62.1
	B	nd			nd			65.9	±	6.8	2.3	±	0.1	nd			17.3	±	1.4	nd			83.6
	C	nd			nd			123.1	±	1.7	1.9	±	0.1	nd			19.7	±	0.5	nd			143.4
Chicory/Escarole	A	nd			nd			6.1	±	1.2	nd			nd			nd			nd			6,7
	B	nd			nd			25.4	±	0.6	nd			nd			nd			nd			26.0
	C	nd			nd			3.0	±	1.0	nd			nd			nd			nd			3.8
Collard greens	A	nd			136.3	±	11.6	nd			nd			nd			nd			nd			140.5
	B	nd			89.0	±	3.7	nd			nd			nd			nd			nd			88.5
	C	nd			47.9	±	0.8	nd			nd			nd			nd			nd			47.1
Spinach	A	2.4	±	0.2	nd			nd			nd			nd			nd			nd			2.6
	C	nq			nd			nd			nd			nd			nq			nd			0.0
	D	5.3	±	0.7	nd			nd			nd			nd			nd			nd			6.1
Mustard	A	nd			nd			nd			nd			149.1	±	2.1	8.0	±	0.3	nd			159.7
	B	nd			nd			nd			nd			190.8	±	2.2	1.2	±	0.1	nd			194.1
Cabbage	A	nd			nd			6.6	±	0.4	nd			nd			nd			nd			6.1
	B	nd			nd			4.4	±	0.2	nd			nd			nd			nd			4.6
	C	nd			nd			2.4	±	0.3	nd			nd			nd			nd			2.1

Vegetable	Supplier	CAFFEIC			3-CQA			5-CQA			4-CQA			3.4-DQA			3.5-DQA			4.5-DQA			Sum
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Purple Garlic	A	nd			nd			nd			nd			nd			nd			nd			0.0
	B	nd			nd			nd			nd			nd			nd			nd			0.0
	C	nd			nd			nd			nd			nd			nd			nd			0.0
Arugula	A	nd			nd			nd			nd			1.7	±	0.0	22.3	±	1.5	36.6	±	1.0	61.6
	B	nd			nd			nd			nd			1.5	±	0.2	25.5	±	1.6	24.4	±	2.4	47.7
	D	nd			nd			nd			nd			11.6	±	0.3	31.9	±	1.2	19.9	±	1.7	61.0
Fruits																							
Japanese Pumpkin	A	nd			nd			nd			nd			nd			0.4	±	0.0	nd			0.4
	B	nd			nd			nd			nd			nd			0.2	±	0.0	nd			0.2
	C	nd			nd			nd			nd			nd			0.7	±	0.1	nd			0.6
Italian Zucchini	A	nd			nd			nd			nd			1.1	±	0.1	0.3	±	0.0	nd			1.4
	B	nd			nd			nd			nd			1.0	±	0.2	0.2	±	0.0	nd			1.4
	C	nd			nd			nd			nd			3.2	±	0.2	nq			nd			3.0
Eggplant	A	nd			nd			31.9	±	3.5	3.2	±	0.3	nd			nd			nd			38.4
	B	nd			nd			81.8	±	10.2	2.5	±	0.1	nd			nq			nd			93.8
	C	nd			nd			31.8	±	5.2	2.3	±	0.2	nd			0.2	±	0.0	nd			38.4
Caxi/edible Porongo	A																						0.0
Chayote	A	nd			nd			nd			nd			nd			nd			nd			0.0
	B	nd			nd			nd			nd			nd			nd			nd			0.0
	C	nd			nd			nd			nd			nd			nd			nd			0.0
Scarlet Eggplant	B	nd			nd			20.8	±	1.5	nd			nd			nd			1.3	±	0.1	22.6
	D	nd			nd			9.1	±	0.4	nd			nd			nd			1.7	±	0.1	11.1
	E	nd			nd			19.5	±	2.6	nd			nd			nd			1.7	±	0.1	18.3
Cackrey	A	nd			nd			nd			nd			nd			0.3	±	0.0	nd			0.3
	B	nd			nd			nd			nd			nd			0.4	±	0.1	nd			0.3
	C	nd			nd			nd			nd			nd			0.1	±	0.0	nd			0.1
Bitter Melon	A	nd			nd			nd			nd			nd			nd			0.3	±	0.1	0.2

Vegetable	Supplier	CAFFEIC	3-CQA	5-CQA	4-CQA	3.4-DQA	3.5-DQA	4.5-DQA	Sum
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Japanese cucumber	A	nd	nd	nd	0.9 ± 0.1	nd	nd	nd	0.8
	B	nd	nd	nd	1.2 ± 0.0	nd	nd	nd	1.2
	C	nd	nd	nd	nd	nd	nd	nd	0.0
Green pepper	A	nd	nd	1.0 ± 0.1	nd	nd	nd	nd	1.1
	B	nd	nd		nd	nd	nd	nd	0.0
	C	nd	nd	1.5 ± 0.3	nd	nd	nd	nd	1.8
Okra	A	nd	3.9 ± 0.2		nd	nd		nd	3.6
	B	nd	4.8 ± 0.3	1.6 ± 0.2	nd	nd	1.4 ± 0.1	nd	7.3
	C	nd	21.6 ± 4.0		nd	nd	1.1 ± 0.1	nd	27.0
Stems									
Asparagus	A	nd	nd	nd	24.6 ± 0.7	nd	nd	nd	25.2
	B	nd	nd	nd	33.8 ± 2.7	nd	nd	nd	34.7
	C	nd	nd	nd	38,2 ± 1.9	nd	nd	nd	36.0
Celery	A	nd	nd	96.6 ± 14.8	nd	91.4 ± 10.0	nd	nd	186.8
	B	nd	nd	42.1 ± 5.1	nd	155.6 ± 5.6	nd	nd	209.3
	C	nd	nd	18.9 ± 0.2	nd	109.1 ± 8.5	nd	nd	137.6
Leguminous plants									
Fresh Pea	A	nd	nd	nd	nd	nd	nd	nd	0.0
	B	nd	nd	nd	nd	nd	nd	nd	0.0
	C	nd	nd	nd	nd	nd	nd	nd	0.0
Green bean	A	nd	nd	nd	nd	nd	2.9 ± 0.5	nd	3.2
	B	nd	nd	nd	nd	nd	1.4 ± 0.2	nd	1.5
	C	nd	nd	nd	nd	nd	3.9 ± 0.4	nd	3.5
Lablab-bean	A	nd	nd	nd	nd	0.6 ± 0.1	nd	nd	0.7
	B	nd	nd	nd	nd	0.4 ± 0.1	nd	nd	0.3
	C	nd	nd	nd	nd	0.4 ± 0.1	nd	nd	0.5

Vegetable	Supplier	CAFFEIC	3-CQA	5-CQA	4-CQA	3.4-DQA	3.5-DQA	4.5-DQA	Sum
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Roots									
Pink sweet potato	D	nd	nd	13.7 ± 2.0	nd	0.2 ± 0.0	4.5 ± 0.7	nd	21.1
	E	nd	nd	18.7 ± 2.5	nd	2.7 ± 0.2	10.1 ± 1.8	nd	33.8
	F	nd	nd	2.7 ± 0.4	nd		1.3 ± 0.1	nd	4.3
Beets	A	nd	nd	nd	nd	nd	nd	nd	0.0
	B	nd	nd	nd	nd	nd	nd	nd	0.0
	C	nd	nd	nd	nd	nd	nd	nd	0.0
Carrot	A	nd	0.3 ± 0.0	nq	nd	nd	nd	nd	0.2
	B	nd	0.1 ± 0.0	nq	nd	nd	nd	nd	0.1
	C	nd	0.4 ± 0.0	0.9 ± 0.1	nd	nd	nd	nd	1.2
Cassava	A	nd	nd	nd	nd	nd	nd	nd	0.0
	B	nd	nd	nd	nd	nd	nd	nd	0.0
	C	nd	nd	nd	nd	nd	nd	nd	0.0
Arracacha	A	nd	nd	6.3 ± 0.4	nd	nd	nd	nd	6.2
	B	nd	nd	8.1 ± 0.1	nd	nd	nd	nd	8.1
	C	nd	nd	4.0 ± 0.5	nd	nd	nd	nd	4.1
Turnip	A	nd	nd	nd	nd	nd	nd	nd	0.0
	B	nd	nd	nd	nd	nd	nd	nd	0.0
	C	nd	nd	nd	nd	nd	nd	nd	0.0
Radish	A	nd	nd	nd	nd	4.2 ± 0.1	nd	0.7 ± 0.1	5.0
	B	nd	nd	nd	nd	4.9 ± 0.1	nd	0.4 ± 0.0	5.1
	C	nd	nd	nd	nd	3.9 ± 0.1	nd	0.6 ± 0.1	4.3
Rhizomes									
Ginger	A	nd	nd	nd	nd	nd	nd	nd	0.0
	B	nd	nd	nd	nd	nd	nd	nd	0.0
	C	nd	nd	nd	nd	nd	nd	nd	0.0

Vegetable	Supplier	CAFFEIC			3-CQA			5-CQA			4-CQA			3.4-DQA			3.5-DQA			4.5-DQA			Sum
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Purple Yam	A	nd			nd			nd			nd			nd			nd			nd			0.0
	B	nd			nd			nd			nd			nd			nd			nd			0.0
	C	nd			nd			nd			nd			nd			nd			nd			0.0
Tubers																							
English potato	A	0.5	±	0.0	nd			0.9	±	0.1	nd			nd			nq			0.3	±	0.0	1.7
	B	0.3	±	0.0	nd			3.3	±	0.2	nd			nd			nq			0.3	±	0.0	4.1
	C	0.5	±	0.1	nd			2.9	±	0.2	nd			nd			0.1	±	0.0	0.4	±	0.1	3.6
Yam	A	nd			nd			nq			nd			nd			nd			nd			0.0
	B	nd			nd			nq			nd			nd			nd			nd			0.0
	C	nd			nd			nd			nd			nd			nd			nd			0.0

Caffeic: caffeic acid; 3-CQA: 3-caffeoylquinic acid; 5-CQA: 5-caffeoylquinic acid; 4-CQA: 4-caffeoylquinic acid; 3.4-DQA: 3.4 dicaffeoylquinic acid; 3.5-DQA: 3.5-dicaffeoylquinic acid; 4.5-DQA: 4.5-dicaffeoylquinic acid; Average: obtained by triplicate of analysis; Results expressed as wet basis; **SD**: Standard deviation; Absence of values indicates that the compound was not detected; **nq**: values between the limit of detection and quantification (0.005 mg/kg^{-1} and 0.01 mg/kg^{-1}), respectively; **nd**: indicate that the compound was not detected.

3.3.2. Chlorogenic and cafeic acids

Figure 2 shows the vegetables that had majority concentrations of the studied compounds.

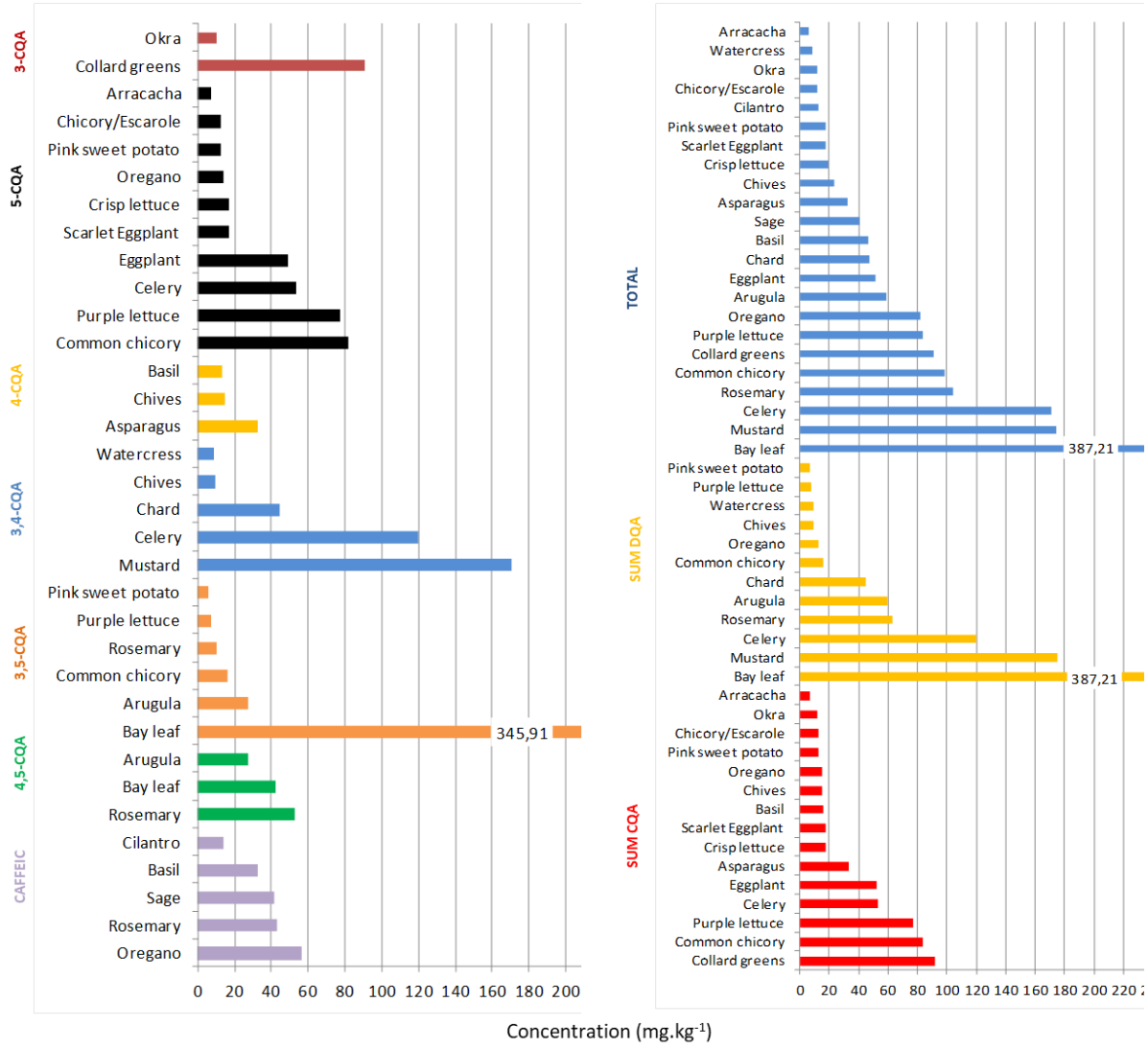


Figure 2 Vegetables with highest concentration of the studied compounds

Herein, 3-CQA was found in only 3 vegetables: collard greens, okra and carrots, with average values between suppliers of 91.1 $mg.kg^{-1}$, 10.1 $mg.kg^{-1}$ and 0.3 $mg.kg^{-1}$, respectively. Fiol et al., (2012) carried out the identification of 3-CQA in collard greens without, however, quantifying it. The other samples in which this analyte was detected had not been reported previously.

Among the compounds evaluated in this study, 5-CQA is the chlorogenic acid most reported in literature concerning vegetables (BARROS et al., 2012; CHEN; KANG, 2013;

YOON; CHUNG; THIRUVENGADAM, 2015) and was found in 55 samples among the 153 studied, with values between 0.01 and 128.0 $mg.kg^{-1}$. The highest levels were in common chicory, purple lettuce, celery and eggplant, with average values between suppliers, respectively, from 80.9, 76.6, 52.5 and 48.5 $mg.kg^{-1}$. Papetti et al. (2017) and Sinkovic et al. (2015) only identified 5-CQA in the sample of common chicory, while Heimler et al. (2009) quantified 5-CQA in the same vegetable in higher concentrations (120 $mg.kg^{-1}$). Mattila and Hellstrom (2007) and Galieni et al. (2015) studied purple lettuce and found a value for 5-CQA of 4.2 $mg.kg^{-1}$ (frozen lettuce) and 510 $mg.kg^{-1}$ (fresh samples), and both diverged from values found in this study. Celery was studied by Neacsu et al. 92015), however, lower concentrations were observed in their study (9.6 -1 $mg.kg^{-1}$) compared with the current study. Mattila and Hellstrom (2007) obtained a value of 310 $mg.kg^{-1}$ of 5-CQA for eggplant, whereas Kaushik et al. (2017) found it to be 265.7 $mg.kg^{-1}$.

Herein, 4-CQA was detected in 11 vegetables and varied between 0.1 and 38.2 $mg.kg^{-1}$, with asparagus samples (32.2 $mg.kg^{-1}$), chives (13.7 $mg.kg^{-1}$) and basil (12.8 $mg.kg^{-1}$) having the highest average levels. None of these samples have previously been reported in the literature concerning identification and quantification of this compound.

Herein, 5-CQA was the major compound between the monocaffeoylquinic acids, both in concentration and number of detected samples. When considering the combination of all monocaffeoylquinic acids, collard greens (91.1 $mg.kg^{-1}$), common chicory (82.8 $mg.kg^{-1}$), purple lettuce (76.6 $mg.kg^{-1}$), celery (52.5 $mg.kg^{-1}$) and eggplant (51.2 $mg.kg^{-1}$) were the vegetables with the highest average concentrations.

With regard to dicaffeoylquinic acids, 3,4-DQA was quantified in 28 samples, with concentrations between 0.2 and 190.8 $mg.kg^{-1}$. Samples of mustard, celery and chard presented higher average quantities than the others, with 169.9, 118.7 e 44.0 $mg.kg^{-1}$, respectively, followed by samples of chives (9.0 $mg.kg^{-1}$) and basil (7.6 $mg.kg^{-1}$). In the literature, only studies that report the presence of 3,4-DQA for some samples of vegetables were found. Esatbeyoglu et al. (2016), obtained a 109.0 $mg.kg^{-1}$ content in sweet potato, while only the identification of the compound was made in common chicory (PAPETTI et al., 2017) and purple yam (CHAMPAGNE et al., 2011). For rosemary and sage, the investigation of this compound in dehydrated samples was performed without finding detectable values (MEINHART et al., 2017a).

Herein, 3,5-DQA was present in 49 samples out of 153, with values between 0.01 and 362.3 $mg.kg^{-1}$. Bay leaf presented the highest average concentration with 345.9 $mg.kg^{-1}$, followed by arugula with 26.6 $mg.kg^{-1}$. Moreover, common chicory (15.3 $mg.kg^{-1}$), rosemary

(9.5 mg.kg^{-1}), purple lettuce (6.9 mg.kg^{-1}), and sweet potato (5.3 mg.kg^{-1}) can be cited with significant amounts of 3,5-CQA. Among the samples that had significant levels, sweet potato had been presented in prior studies by (Esatbeyoglu et al. (2016) (266.0 mg.kg^{-1}) and (ZHENG; CLIFFORD, 2008) (only identification by mass spectrometry), as well as rosemary, for which (MEINHART et al., 2017a) found 124.0 mg.kg^{-1} in dehydrated samples.

As for 4,5-DQA, values between 0.3 and 62.9 mg.kg^{-1} were found in 24 samples. Rosemary, bay leaves, arugula and oregano were among the samples with highest average concentrations, 52.3, 41.3, 27.0 and 9.2 mg.kg^{-1}, respectively. None of these samples had been studied in the literature evaluating this compound, except for rosemary, where 8460.6 mg.kg^{-1} was quantified in dehydrated samples, a value that is greater than that found in this study (MEINHART et al., 2017a).

Herein, 3,5-DQA was the most abundant compound found among the dicaffeoylquinic acids in prevalence and concentration, followed by 3,4-DQA. Considering all of the dicaffeoylquinic acids, these were present in 25 plants, at concentrations between 0.1 and 387.2 mg.kg^{-1}. The vegetables that had higher average concentrations were bay leaf (387.2 mg.kg^{-1}), mustard (174.6 mg.kg^{-1}), celery (118.7 mg.kg^{-1}), rosemary (61.9 mg.kg^{-1}), arugula (58.5 mg.kg^{-1}) and chard (44.0 mg.kg^{-1}). Only 7 of the 53 studied vegetable species had already been reported in the literature regarding dicaffeoylquinic acids, including eggplant, yams, sweet potatoes, common chicory, rosemary and sage (CHAMPAGNE et al., 2011; ESATBEYOGLU et al., 2016; MEINHART et al., 2017a; PAPETTI et al., 2017; WU et al., 2013; ZHENG; CLIFFORD, 2008).

Caffeic acid was found only in 22 samples, at concentrations between 0.01 and 74.7 mg.kg^{-1}. Samples of oregano (55.1 mg.kg^{-1}), rosemary (42.2 mg.kg^{-1}), sage (41.0 mg.kg^{-1}) basil (32.0 mg.kg^{-1}) and cilantro (12.9 mg.kg^{-1}) contained higher average concentrations. Caffeic acid has not been previously investigated in oregano, but it have been observed in basil and cilantro; this analyte was only identified by Antora and Salleh (2017), Grădinariu et al. (2013) and El-Zaeddi et al. (2017). Nevertheless, Meinhart et al. (2017) performed the quantification in dried sage. And Maldini et al. (2016) performed the quantification in rosemary (ranging from 34.6 to 720.6 mg.kg^{-1}).

When considering both chlorogenic and caffeic acids, among 113 samples that had some quantity present, bay leaf, celery, rosemary, common chicory, collard greens, purple lettuce, oregano and arugula presented the highest amounts, ranging between 58.5 and 387.2 mg.kg^{-1}.

Among the suppliers, significant variations have also been observed, such as in sweet potato, purple lettuce, collard greens and common chicory, which had variations in relative standard deviation of 76.2%, 59.5%, 48.6% and 42.3%, respectively, when all compounds were considered. These variations may be explained by differences in soil and climate conditions to which these samples were exposed during their cultivation (KIM et al., 2015).

3.3.3. Concentration of compounds

The frequency and distribution of concentration of analytes in the studied samples in relation to the botanical classification can be seen in Figure 3. It was observed that the vegetables including sprouts, bulbs, rhizomes and tubers did not show significant concentrations of all compounds. On the other hand, the condiments, leaf vegetables, fruits and stems were the groups that contributed the highest concentrations. The highest concentration of 3-CQA was in the leaf vegetable sample, whereas the largest concentrations of 4-CQA were observed in condiments and stems. The highest concentration of 5-CQA was found in condiments, flowers and leguminous plants; the highest concentration of 3,4-DQA was found in leaf vegetables and stems; the highest concentration of 3,5-DQA was found in condiments, leaf vegetables and roots,; the highest concentration of 4,5-DQA was found in condiments and leaf vegetables; and the highest concentration of caffeic acid was found in condiments.

4. CONCLUSION

In this study, the concentrations of six isomers of chlorogenic acid and caffeic acid were investigated in 53 vegetables sold and consumed in several regions of Brazil. Among the samples that stood out regarding all levels of monocaffeoylquinic acids (3-CQA, 4-CQA and 5-CQA) are the collard greens, common chicory and purple lettuce, whereas the highest concentrations of the dicaffeoylquinic acids (3,4-DQA, 3,5-DQA and 4,5-DQA) were found in bay leaf, mustard and celery. Oregano, sage and rosemary had the highest concentrations of caffeic acid. When considering the total of all evaluated compounds, the most relevant samples are classified as condiments and leaf vegetables. Among the studied samples, only the sweet potatoes, rosemary, sage and common chicory had been previously reported in relation to the presence of these six isomers and caffeic acid; thus, this study provides new information about these bioactive compounds and identifies new sources of these isomers.

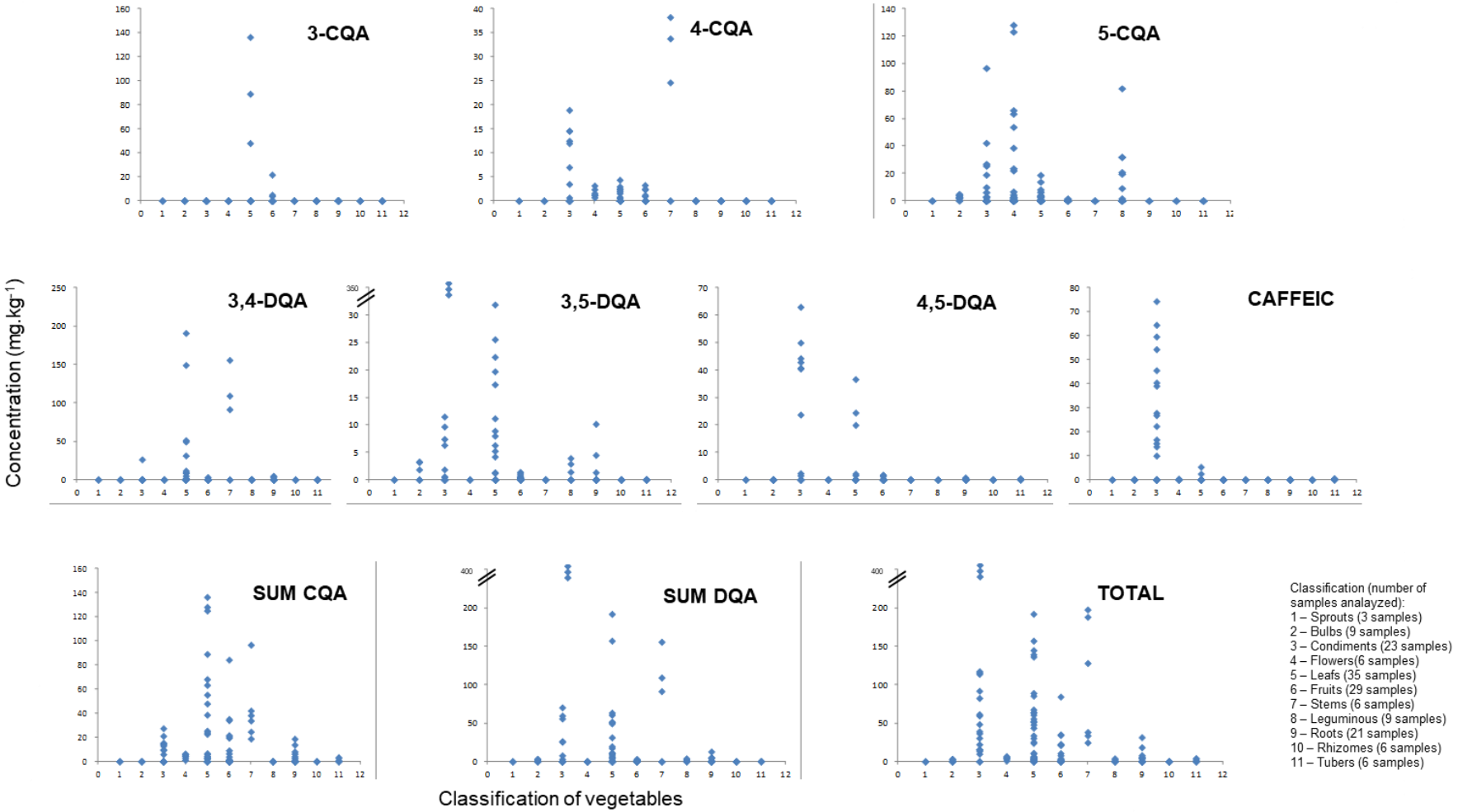


Figure 3 Frequency and distribution of concentration of analytes in the studied samples in relation to the botanical classification

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CAPÍTULO IV

DETERMINAÇÃO DE RUTINA EM FRUTAS E HORTALIÇAS *IN NATURA*

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RESUMO

A rutina é um dos compostos fenólicos que vem despertando interesse devido ao efeito benéfico na redução do risco de doenças degenerativas, sendo assim, é de extrema importância investigar novas fontes naturais desse composto. O objetivo deste estudo foi avaliar o teor de rutina nas partes comestíveis de 324 amostras de vegetais, compreendendo 117 diferentes frutas e hortaliças comercializadas no Brasil. A rutina foi detectada em 73 vegetais diferentes (195 amostras). Umbu, noni, amora, marmelo e cereja foram as frutas com as maiores concentrações de rutina (entre 43,2 e 162,4 $mg.kg^{-1}$). Dentre as hortaliças, os maiores teores encontrados foram em coentro (196,6 $mg.kg^{-1}$) e aspargo (151,3 $mg.kg^{-1}$). Considerando as porções normalmente ingeridas, os vegetais que apresentaram maior quantidade foram umbu, aspargo, amora, marmelo, cereja e ameixa roxa, e, podem ser consideradas como fontes de rutina para uma dieta diversificada e saudável.

Palavras chave: compostos fenólicos, flavonoides, vegetais, cromatografia líquida.

1. INTRODUÇÃO

Ultimamente, muitos estudos relacionam compostos bioativos presentes em frutas e hortaliças aos efeitos benéficos à saúde, com maior ênfase na redução do risco de doenças degenerativas (GELEN et al., 2017; HUANG et al., 2017a; MILLER et al., 2017; TORRES-RÊGO et al., 2016). Dentre esses, os mais estudados são os compostos fenólicos, oriundos do metabolismo secundário das plantas (GULLÓN et al., 2017; SHAHIDI; AMBIGAIPALAN, 2015). Sua produção é estimulada pelos mecanismos de defesa em relação às condições de estresse (DIAS et al., 2016; MATKOWSKI, 2008).

A rutina é um flavonoide, da classe dos flavonóis, formado por uma molécula de quercetina e ramnose ligadas no carbono três (GHORBANI, 2017), sintetizada através da via do fenilpropanoide, onde há a transformação de fenilalanina em 4-cumaroil-coenzima A, seguida de ação enzimática (FAGGIO et al., 2017; SELEEM; PARDI; MURATA, 2017). É um dos flavonoides de maior importância na indústria farmacêutica, estando presente em formulações medicinais e terapêuticas patenteadas em vários países (CHUA, 2013; GULLÓN et al., 2017; SHARMA et al., 2013).

Tem sido atribuída à rutina uma elevada capacidade antioxidante, além de apresentar atividades biológicas importantes (ABARIKWU et al., 2017; HSU et al., 2009; PANCHAL et al., 2011; PRINCE; KAMALAKKANNAN, 2006). Estudos apontam que ratos diabéticos suplementados com esse flavonoide apresentaram efeito de melhora no estado glicêmico (HAO et al., 2012). Em outra pesquisa também foi verificada eficiência na redução dos níveis de glicose sanguínea e na pressão arterial sistólica e diastólica (SATTANATHAN et al., 2011). Efeitos na redução da hipertrofia do miocárdio, aliviando a deposição de colágeno e o acúmulo de lipídios (HUANG et al., 2017a), bem como ação anti-inflamatória no tratamento de colite, peritonite e redução de edemas e citoquinas também foram relacionados ao consumo de rutina (RABIŠKOVÁ et al., 2012; TORRES-RÊGO et al., 2016).

Estudos *in vivo* e *in vitro* demonstraram efeitos anticarcinogênicos da rutina, indicando causar a redução no ciclo celular e induzir a apoptose em células cancerígenas (PERK et al., 2014). Karakurt (2016) evidenciou a ação antiproliferativa e moduladora do carcinoma hepatocelular humano, enquanto outros trabalhos reportaram efeito quimiopreventivo e antitumorais *in vivo* (ALONSO-CASTRO; DOMÍNGUEZ; GARCÍA-CARRANCÁ, 2013). A rutina também foi relacionada com o alívio da aterosclerose (LIA, 2018), assim como ações protetoras sobre a hepatotoxicidade (GELEN et al., 2017).

A rutina é frequentemente encontrada em fontes vegetais, como frutas, hortaliças e grãos (ATANASSOVA; BAGDASSARIAN, 2009), salsa, framboesa (PAVLOVIĆ et al., 2016; YILDIZ et al., 2008), açaí, ameixa roxa, noni, banana, laranja, goiaba (AMIR et al., 2013; GARZÓN et al., 2017; LIN et al., 2014; OBOH et al., 2015; PANDY et al., 2014), manjericão, almeirão (DALAR; KONCZAK, 2014; FRATIANNI et al., 2016), rabanete, cenoura (OBOH et al., 2015), lentilha (FRATIANNI et al., 2014b) e trigo sarraceno (KIM et al., 2005). Assim, dada a importância da rutina na redução do risco a diversas doenças, a ingestão deste composto pela dieta pode ser fortemente recomendada. Considerando a diversidade de frutas e hortaliças disponíveis no Brasil, e dos benefícios relacionados ao consumo desse flavonoide, esse trabalho teve como objetivo identificar e quantificar a rutina em 117 vegetais *in natura*, buscando evidenciar potenciais fontes naturais.

2. MATERIAIS E MÉTODOS

2.1. AMOSTRAS E REAGENTES

Foram estudados 117 diferentes vegetais, sendo 64 frutas e 53 hortaliças. Cada amostra foi adquirida de três fornecedores distintos (exceto quando não disponível), totalizando 324 amostras. Os fornecedores foram oriundos de 16 estados brasileiros das regiões sudeste, nordeste, sul, norte e centro-oeste do país. Todas as amostras foram adquiridas no Brasil, embora algumas sejam importadas de outros países, como Estados Unidos, Chile, Portugal, Espanha, Colômbia e México. As amostras *in natura* foram obtidas no estádio de maturação considerado apto para o consumo. A quantidade de amostra adquirida, de cada fornecedor foi de 0,5 kg para amostras pequenas (como amora e ameixa, por exemplo) e de 3 unidades para as amostras maiores (quando 0,5 kg não atingia a quantidade de 3 unidades) como melancia, melão, abóbora, entre outros. Para amostras folhosas (como rúcula, salsa e outras) foram adquiridos 3 maços (forma como são comercializados) de cada fornecedor.

O padrão analítico de rutina foi adquirido da Sigma Aldrich (EUA). A solução estoque de padrão de rutina foi preparada em acetonitrila grau cromatográfico (J.T. Baker, Brasil), na concentração de 1 $mg.mL^{-1}$, e armazenada a -80 °C. O ácido fórmico foi adquirido da Merck (Brasil), acetonitrila grau cromatográfico da JT Backer (Brasil) e etanol de grau analítico da Synth (Brasil). A água empregada nos experimentos foi ultrapurificada em sistema Milli-Q® (Millipore, EUA). Todas as soluções foram filtradas em membranas de fluoreto de polivinilideno (PVDF) de porosidade de 0,22 µm (Millipore, EUA).

2.2. PREPARO DAS AMOSTRAS

Após a remoção de sujidades e das partes não comestíveis, as partes comestíveis dos vegetais foram trituradas empregando liquidificador, triturador ou processador até, aproximadamente, 200 *mesh*. Imediatamente após o preparo, as amostras foram submetidas à extração para análise de rutina. As amostras de açaí foram pré-tratadas por imersão em água a 60 ºC durante 60 min e então submetidas ao despolpamento, separadas das sementes e analisadas (considerando o teor de água incorporado).

2.3. MÉTODOS DE ANÁLISE

Todas as amostras (1 g) foram extraídas com 15 mL de água:etanol (74:26) em tubo Falcon® de 50 mL, baseando-se no método descrito por Meinhart et al. (2017). O tubo hermeticamente fechado foi submetido a agitação (240 rotações por minuto) em banho-maria a 60 ºC, por 22 min. Em seguida, a amostra foi filtrada em filtro de papel e o extrato líquido em filtro de membrana de PVDF com porosidade de 0,22 µm. As amostras de avocado, abacate fortuna e côco seco tiveram o procedimento de extração precedido por uma etapa de remoção dos lipídios mediante partição com éter etílico.

A análise de rutina foi realizada por cromatografia líquida de alta eficiência com detector de arranjo de diodos (HPLC-DAD) operando a 325 nm, em um equipamento Agilent Technologies (Alemanha), modelo 1260, equipado com injetor automático e bomba quaternária, coluna C18 Zorbax Eclipse plus (Agilent Technologies, Alemanha), de 4,6 mm de i.d., 100 mm de comprimento e 3,5 µm de tamanho de partícula, mantida sob temperatura de 30 °C, baseando-se método descrito por Meinhart et al. (2017).

A eluição foi conduzida por sistema de gradiente iniciando com 10% de A (acetonitrila) e 90% de B (água acidificada com 0,1% de ácido fórmico, pH 2,4), com variação linear até atingir 40% de A aos 6 min. A partir de 6,1 min chegou-se a 100% de A e foi mantido assim até os 7,5 min para limpeza da coluna em virtude da diversidade das amostras. Em seguida, a coluna foi recondicionada com a composição inicial de fase móvel durante 3,5 min. A vazão da fase móvel foi de 1,2 $mL.min^{-1}$ e o volume de injeção foi de 30 µL. A identificação da rutina foi realizada por comparação com padrão analítico através do tempo de retenção, espectro de absorção do DAD e por co-cromatografia. A quantificação foi realizada por curva de calibração externa. O tratamento estatístico das amostras foi realizado através da análise de variância (ANOVA) e teste de Tukey, com 95% de confiança, através do

software Statistica 7.0 (Statsoft, USA). A igualdade das variâncias foi confirmada através do teste de Cochran (MILLER; MILLER, 2010).

A validação do método foi realizada seguindo as recomendações da IUPAC (THOMPSON; ELLISON; WOOD, 2002) e ANVISA (ANVISA, 2017). Dessa forma, os limites de detecção e quantificação foram estabelecidos como a concentração correspondente a relação de 3 e 6 vezes o sinal/ruído, respectivamente. A faixa linear foi estabelecida em curva analítica construída com 6 pontos equidistantes, em triplicatas aleatórias, iniciando no limite de quantificação e terminando na concentração até onde a linearidade foi assegurada através da avaliação dos modelos quanto a falta de ajuste e significância da regressão segundo a ANOVA realizada através do *software* Statistica 7.0 (Statsoft, USA). A exatidão foi avaliada por ensaios de recuperação em amostra de laranja e brócolis, em três níveis representados pelo limite de quantificação, concentração intermediária e concentração máxima da curva analítica. A precisão no dia foi realizada através de 7 determinações sucessivas em amostras de laranja e brócolis (cada qual em três níveis, idênticos aos da exatidão) e a precisão entre dias através da determinação em 3 diferentes dias, em amostras de laranja e brócolis (cada qual em três níveis, idênticos aos da exatidão), com 7 determinações em cada dia.

3. RESULTADOS E DISCUSSÃO

3.1. VALIDAÇÃO

As figuras de mérito da validação do método analítico estão apresentadas na Tabela 1. Os resultados obtidos mostraram baixos limites de detecção e quantificação (0,008 $mg.kg^{-1}$ e 0,015 $mg.kg^{-1}$, respectivamente), linearidade adequada entre as concentrações de 0,03 e 10 $mg.L^{-1}$ (já que o valor de F para a falta de ajuste foi menor que o F crítico$_{(4,14)}$, com 95% de confiança) e exatidão entre 91,8 a 101,6% considerando os três níveis de recuperação das matrizes de laranja e brócolis. Foi obtido elevada precisão na quantificação das amostras, com desvios padrão relativos menores que 6%, considerando os três níveis estudados (limite de quantificação, ponto intermediário e concentração máxima da curva analítica). Tais resultados estão de acordo com os limites estabelecidos pela IUPAC (THOMPSON; ELLISON; WOOD, 2002) e pela ANVISA (ANVISA, 2017), demonstrando que o método é recomendado para realizar análises quantitativas com segurança analítica.

Tabela 1 Figuras de mérito da validação do método analítico empregado na análise de rutina em vegetais por HPLC-DAD

Parâmetros		Resultados
Faixa Linear da curva analítica (mg.L^{-1})		0,03 a 10,0
Valor F calculado para ajuste do modelo linear[(1)]		0,104
Exatidão (recuperação em amostra de laranja), em % recuperado (n=3)	Nível 1	98,37
	Nível 2	103,58
	Nível 3	100,60
Exatidão (recuperação em amostra de brócolis), em % recuperado (n=3)	Nível 1	101,62
	Nível 2	91,80
	Nível 3	94,41
Precisão no dia (n=7) em amostra de laranja fortificada, em desvio padrão relativo	Nível 1	2,21
	Nível 2	1,25
	Nível 3	1,34
Precisão no dia (n=7) em amostra de brócolis fortificada, em desvio padrão relativo	Nível 1	2,84
	Nível 2	1,96
	Nível 3	2,68
Precisão entre dias (n=3) em amostra de laranja fortificada, em desvio padrão relativo	Nível 1	7,64
	Nível 2	5,48
	Nível 3	1,78
Precisão entre dias (n=3) em amostra de brócolis fortificada, em desvio padrão relativo	Nível 1	5,24
	Nível 2	2,71
	Nível 3	2,68
Limite de Quantificação (mg.kg^{-1})		0,015
Limite de Detecção (mg.kg^{-1})		0,008

(1): O modelo apresenta ajuste adequando quanto o F calculado for menor que o F crítico$_{4,14}$ de 3,11(com 95% de confiança). Nível 1: Limite de quantificação; Nível 2: Concentração intermediária da faixa linear da curva analítica; Nível 3: Concentração máxima da faixa linear da curva analítica.

3.2. RUTINA EM FRUTAS E HORTALIÇAS

Na Tabela 2 estão apresentadas a identificação das amostras, suas origens, partes analisadas, bem como as concentrações de rutina. Esses resultados evidenciaram a presença de rutina em 61% das amostras (195), que apresentaram teor superior ao limite de quantificação (entre 0,3 e 479,6 mg.kg^{-1}). Em 2 amostras o composto foi detectado em

quantidade inferior ao limite de quantificação e em 127 amostras a presença do composto não foi detectada. Os vegetais que apresentaram maior quantidade média de rutina (considerando todos os fornecedores) foram coentro, umbu, aspargo, noni, amora, marmelo e cereja, com concentrações médias de 196,6 $mg.kg^{-1}$, 162,4 $mg.kg^{-1}$, 151,3 $mg.kg^{-1}$, 99,2 $mg.kg^{-1}$, 60,6 $mg.kg^{-1}$,58,3 $mg.kg^{-1}$ e 43,2 $mg.kg^{-1}$, respectivamente. Quando avaliados os 73 vegetais nos quais a rutina foi detectada em um ou mais dos seus fornecedores, em 42% deles foi observada uma variação maior do que 50% (em desvio padrão relativo) na concentração entre os diferentes fornecedores.

Msaada et al. (2017) avaliaram amostras de coentro onde obtiveram valores entre 1,1 a 139,6 $mg.kg^{-1}$ em base secas. A diferença nas concentrações em relação a este trabalho (58,3 a 479,6 $mg.kg^{-1}$ em base úmida ou, considerando o teor de umidade da amostra de 89%, 530,0 a 4360,0 $mg.kg^{-1}$ em base seca) provavelmente estão relacionadas à parte do vegetal que foi analisada, já que os autores citados usaram os frutos do coentro ao invés de folhas e talos. Em ambos os trabalhos foram observadas diferenças consideráveis entre os fornecedores atribuídas à proveniência de localidades distintas.

O umbu apresentou a segunda maior concentração média de rutina entre os vegetais analisados e, ao que consta aos autores, foi a primeira vez que esse analito foi identificado e quantificado nesta fruta. O aspargo foi estudado por Solana et al. (2015) que obtiveram resultados de 100,0 a 2.810,0 $mg.kg^{-1}$ em base seca, próximos aos encontrados neste trabalho (48,4 a 172,1 $mg.kg^{-1}$ em base úmida ou, considerando 94% de umidade, 806,7 a 2868,3 $mg.kg^{-1}$ em base seca).

Pandy et al. (2014) e quantificaram rutina em noni produzidos na Malásia, obtendo resultados de 1,66 $mg.kg^{-1}$ em base seca. Esses valor foi inferior aos que foram encontrados no presente estudo (30,8 a 143,6 $mg.kg^{-1}$ em base úmida ou, considerada a umidade de 88%, 256,7 a 1196,7 $mg.kg^{-1}$ em base seca), divergências que podem ser atribuídas a fatores como, condições de extração, o cultivar, o grau de maturação, o clima e a localização de cultivo do vegetal (GULLÓN et al., 2017).

A rutina foi determinada também em amoras da Turquia por Gundogdu et al. (2011) que encontraram uma concentração média de 1.423,0 $mg.kg^{-1}$, valores superiores aos do presente trabalho (17,0 a 139,4 $mg.kg^{-1}$) para amoras cultivadas no Brasil. As condições de cultivos das amoras como, por exemplo, uso de fertilizantes, irrigação, além de condições ambientais intrínsecas à localidade (luz, temperatura, nutrientes) bem como variedades distintas, provavelmente são responsáveis por tais diferenças (SKOULA; ABBES; JOHNSON, 2000).

Para as amostras de marmelo e cereja, foram encontrados trabalhos prévios desenvolvidos por Stojanović et al. (2017) e Sotelo et al. (2018), respectivamente. Os valores para o marmelo de distintas regiões da Sérvia variaram entre 126,4 $mg.kg^{-1}$ e 259,9 $mg.kg^{-1}$, superiores aos verificados nas amostras do Brasil estudadas neste trabalho (48,0 a 68,5 $mg.kg^{-1}$). Já para a cereja, oriunda da Nova Zelândia, os autores encontraram concentrações entre 3,0 e 8,1 $mg.kg^{-1}$, valores significativamente inferiores aos obtidos neste estudo (42,2 a 45,1 $mg.kg^{-1}$).

Tabela 2 Identificação das amostras e teor de rutina em base úmida

Nome Popular (Nome Científico)	Origem do Fornecedor	Partes analisadas	Rutina ($mg.Kg^{-1}$)		
			Média	DP	Média entre fornecedores
Abacate fortuna *(Persea americana)*	1	Polpa	-		-
	1		-		
	1		-		
Abacaxi pérola *(Ananas comosus L. Merril)*	1	Polpa	27,9 [a]	0,2	15,0±11,1
	1		8,7 [b]	0,1	
	1		8,5 [b]	0,4	
Abiu (*Pouteria caimito)*	1	Polpa	-		-
	1		-		
	1		-		
Abóbora cabotiá (*Cucurbita moschata Duch (abóbora) x Cucurbita máxima Duch (moranga)*)	1	Polpa	3,1 [b]	0,5	3,8±1,5
	1		5,4 [a]	0,2	
	1		2,7 [b]	0,4	
Abricó *(Mammea american)*	2	Polpa	1,5 [a]	0,2	1,8±0,5
	2		2,1 [a]	1,4	
Açaí *(Euterpe olearacea Mart)*	2	Polpa	-		-
Atemóia *(Annona cherimola Mill x Annona squamosa L)*	1	Polpa	4,7 [a]	0,1	4,5±0,4
	1		4,0 [a]	0,5	
	1		4,7 [a]	0,1	
Avocado *(Persea americana* var. Hass e Fuerte*)*	1	Polpa	-		-
	1		-		
	3		-		
Cacau (*Theobroma cacao)*	1	Polpa	3,8 [ab]	0,3	3,9±0,8
	2		3,1 [b]	0,1	
	4		4,7 [a]	0,5	
Cajamanga *(Spondias dulcis Som)*	1	Polpa	-		-
	1		-		
	1		-		
Cebola Nacional (*Allium cepa L.*)	3	Polpa	1,6 [a]	0,1	1,0±0,9
	3		-		
	4		1,5 [a]	0,2	

Table 2 (cont.)

Nome Popular (Nome Científico)	Origem do Fornecedor	Partes analisadas	Rutina (mg.Kg^{-1})		
			Média	DP	Média entre fornecedores
Cana de Açúcar *(Saccharum officinarum)*	1	Polpa	-		-
Cupuaçú *(Theobroma grandiflorum)*	4 2	Polpa	1,6 a 0,6 b	0,2 0,1	1,1±0,7
Chuchu (*Sechium edule Sw*)	1 1 1	Polpa	- - -		-
Côco Seco *(Cocos nucifera)*	4 4 4	Polpa	- - -		-
Fruta do conde *(Annona squamosa)*	4 4 4	Polpa	- - -		-
Granadilla *(Passiflora ligularis)*	5 5	Polpa	- -		-
Graviola (*Annona muricata*)	4 4 4	Polpa	15,4 ^{b} 26,1 ^{a} 20,5^{ab}	1,7 2,2 0,6	20,7±5,4
Jabuticaba *(Plinia cauliflora)*	1	Polpa	3,5	0,2	3,5±0,2
Jaca Dura *(Artocarpus integrifolia L.)*	1 1 4	Polpa	2,3 ^{a} - 1,4 ^{b}	0,0 0,1	1,2±1,2
Jatobá *(Hymenaea courbaril)*	4 4 2	Polpa	- - -		-
Jenipapo *(Genipa americana)*	2 2 4	Polpa	7,4 ^{b} 15,1 ^{a} 1,3 ^{c}	0,3 0,9 0,1	7,9±6,9
Laranja pera *(Citrus sinensis)*	1 1 1	Polpa	10,5 ^{a} 7,0 ^{b} 7,0 ^{b}	0,1 0,1 0,2	8,2±2,0
Lima da pérsia *(Citrus limettioides)*	1 1 1	Polpa	1,9 ^{a} 2,1 ^{a} 1,4 ^{b}	0,1 0,1 0,1	1,8±0,4
Limão galego *(Citrus aurantifolia)*	1 1 1	Polpa	11,5 ^{b} 10,9 ^{b} 14,6 ^{a}	0,5 0,3 0,4	12,4±2,0
Limão Taiti *(Citrus aurantifolia)*	1 1 1	Polpa	2,6 ^{ab} 2,2 ^{b} 3,1 ^{a}	0,2 0,0 0,2	2,6±0,5
Mamão Formosa *(Carica papaya L)*	4 1 1	Polpa	2,7 ^{b} 2,3 ^{b} 4,0 ^{a}	0,3 0,1 0,2	3,0±0,9
Manga Palmer *(Mangifera indica)*	4 1 4	Polpa	1,3 ^{b} 2,3 ^{a} 1,8 ^{ab}	0,2 0,1 0,2	1,8±0,5
Mangostão *(Garcinia mangostana)*	2	Polpa	11,6	0,8	11,6±0,8

Table 2 (cont.)

Nome Popular (Nome Científico)	Origem do Fornecedor	Partes analisadas	Rutina (mg.Kg^{-1})		
			Média	DP	Média entre fornecedores
Melancia Crimson *(Citrullus lanatus)*	6 6 4	Polpa	5,8 [b] 9,1 [a] 9,9 [a]	0,1 0,3 0,4	8,2±2,2
Melão Amarelo *(Cucumis melo L.)*	4 4 4	Polpa	- - -		-
Pêra Portuguesa *(Pyrus communis)*	7 7 7	Polpa	- - -		-
Pupunha (*Bactris gasipaes)*	2	Polpa	-		-
Rambutão *(Nephelium lappaceum)*	1 3 1	Polpa	0,6 [c] 6,7 [b] 11,0 [a]	0,1 0,3 1,3	6,1±5,2
Romã *(Punica granatum)*	1 1 1	Polpa	- - -		-
Sapoti *(Manilkara acharas)*	2 2 4	Polpa	- - 1,5	 0,1	0,5±0,9
Tamarindo *(Tamarindus indica L.)*	1 4 4	Polpa	4,9 [a] 4,7 [a] 5,1 [a]	0,3 0,3 0,5	4,9±0,2
Tangerina *(Citrus reticulata)*	1 1 1	Polpa	3,0 [c] 13,2 [a] 4,7 [b]	0,2 1,5 0,3	7,0±5,5
Toranja (*Citrus paradisi)*	8 9	Polpa	- -		-
Caju *(Anacardium occidentale)*	4 4 2	Polpa,casca	1,6 [a] 2,0 [a] 1,5 [a]	0,2 0,2 0,1	1,7±0,2
Carambola Doce (*Averrhoa carambola*)	1 1 1	Polpa,casca	- - -		-
Caqui Ramaforte *(Diosyrus kaki*)	1 1 1	Polpa,casca	1,3 [a] Nq 0,4 [b]	0,1 nq 0,1	0,6±0,7
Kinkan *(Fortunella)*	1 1 1	Polpa,casca	- - -		-
Ameixa Roxa *(Prunus domestica L)*	9 9 11	Polpa,casca	21,0 [b] 32,4 [a] 23,2 [b]	0,6 0,4 1,7	25,5±6,0
Cereja *(Prunus avium)*	9 11 9	Polpa,casca	42,2 [a] 42,2 [a] 45,1 [a]	3,1 1,1 1,6	43,2±1,7

Table 2 (cont.)

Nome Popular (Nome Científico)	Origem do Fornecedor	Partes analisadas	Rutina (mg.Kg^{-1})		
			Média	DP	Média entre fornecedores
Maçã Fugi *(Malus Communis)*	3	Polpa,casca	3,4 ^{b}	0,5	6,4±6,3
	3		13,6 ^{a}	1,3	
	3		2,3 ^{b}	0,1	
Marmelo (*Cydonia oblonga)*	1	Polpa,casca	68,5 ^{a}	11,7	58,3±14,5
	11		48,0 ^{a}	3,9	
Maxixe (*Cucumis anguria L*)	1	Polpa,casca	-		-
	1		-		
	1		-		
Nectarina Garapa *(Prunus Persica)*	8	Polpa,casca	4,1 ^{a}	0,3	3,3±0,7
	8		2,9 ^{b}	0,3	
	10		2,8 ^{b}	0,1	
Noni (*Morinda citrifolia)*	3	Polpa,casca	143,6^{a}	11,5	99,2±60,1
	4		30,8^{b}	3,9	
	1		123,1^{a}	9,5	
Pêssego Sugar Time *(Prunus persica)*	8	Polpa,casca	1,6 ^{b}	0,1	2,6±1,4
	10		2,1 ^{b}	0,2	
	8		4,2 ^{a}	0,1	
Pimentão verde (*Capsicum annuum L.*)	1	Polpa,casca	-		-
	1		-		
	1		-		
Pitanga *(Eugenia uniflora)*	1	Polpa,casca	3,7	0,4	3,7±0,4
Tomate Caeté *(Lycopersicon esculentum)*	1	Polpa,casca	3,1 ^{b}	0,2	5,6±3,2
	1		4,4 ^{b}	0,5	
	1		9,2 ^{a}	0,9	
Umbu (*Spondias tuberosa)*	3	Polpa,casca	288,8^{a}	11,7	162,4±110,2
	1		86,1 ^{b}	10,7	
	4		112,5^{b}	12,4	
Uva Crimson *(Vitis vinifera)*	10	Polpa,casca	2,7 ^{a}	0,2	2,5±0,9
	4		3,2 ^{a}	0,4	
	10		1,5 ^{b}	0,1	
Caxi / Porongo comestível (*Cucurbita* sp.)	1	Polpa,semente	-		-
Banana Nanica *(Musa paradisiaca)*	1	Polpa,semente	0,5 ^{b}	0,0	1,4±1,4
	4		0,5 ^{b}	0,0	
	3		3,0 ^{a}	0,5	
Kiwi *(Actinidia deliciosa)*	3	Polpa,semente	1,5 ^{b}	0,2	3,0±1,2
	3		3,8 ^{a}	0,7	
	3		3,6 ^{a}	0,7	
Maracujá Azedo *(Passiflora edulis Sims)*	1	Polpa,semente	-		-
	1		-		
	1		-		
Melão de Sao Caetano (*Mormodica charantia L.*)	1	Polpa,semente	-		-

Table 2 (cont.)

Nome Popular (Nome Científico)	Origem do Fornecedor	Partes analisadas	Rutina (mg.Kg^{-1})		
			Média	DP	Média entre fornecedores
Pepino japonês (*Cucumis sativus L.*)	1 1 1	Polpa,semente	- - -		-
Pitaya Amarela *(Cereus undatus)*	12 12	Polpa,semente	- -		-
Figo Roxo *(Fícus Carica L.)*	1 1 1	Polpa, casca,semente	21,0 [a] 21,8 [a] 28,6 [a]	2,6 3,0 3,9	23,8±4,2
Framboesa *(Rubus idaeus)*	1	Polpa, casca,semente	2,7	0,4	2,7±0,4
Abobrinha itália (*Cucurbita pepo L.*)	1 1 1	Polpa, casca,semente	- - -		-
Acerola *(Malpighia emarginata DC.)*	2 2 4	Polpa, casca,semente	14,0 [b] 13,2 [b] 40,6 [a]	0,2 0,5 1,4	22,6±15,6
Amora *(Morus nigra)*	1 1 1	Polpa, casca,semente	139,4[a] 17,0 [c] 25,3 [b]	1,8 0,7 2,4	60,6±68,4
Berinjela (*Solanum melongena L.*)	1 1 1	Polpa, casca,semente	1,6 [c] 2,9 [a] 2,4 [b]	0,0 0,1 0,1	2,3±0,7
Goiaba paluma vermelha *(Psidium guajava)*	1 1 1	Polpa, casca,semente	3,1 [a] 2,5 [a] 4,3 [a]	0,4 0,3 0,5	3,3±0,9
Jiló (*Solanum gilo Raddi*)	1 1 1	Polpa, casca,semente	2,4 [a] 2,3 [a] 2,1 [a]	0,4 0,4 0,2	2,3±0,1
Mirtilo (*Vaccinium myrtillus)*	9 1 9	Polpa, casca,semente	22,3 [a] 10,9 [b] 2,1 [c]	1,2 0,5 0,2	11,8±10,2
Morango Albion *(Fragaria X ananassa Duch.)*	1 1 1	Polpa, casca,semente	6,2 [a] 5,7 [a] 5,3 [a]	0,3 0,6 0,1	5,7±0,4
Physalis *(Physalis peruviana)*	3 5 5	Polpa, casca,semente	13,3 [b] 19,7 [a] 11,4 [b]	1,9 2,1 0,5	14,8±4,4
Quiabo (*Abelmoschus esculentus (L.) Moench*)	1 1 1	Polpa, casca,semente	3,7 [ab] 2,5 [b] 6,7 [a]	0,7 0,1 1,1	4,3±2,2
Ervilha Fresca (*Pisum sativum L.*)	1 3 3	Semente	- 2,4 [a] 1,2 [b]	0,1 0,1	1,2±1,2
Vagem (*Phaseolus vulgaris L.*)	1 1 3	Semente,vagem	3,9 [ab] 2,5 [b] 5,7 [a]	0,5 0,2 0,4	4,0±1,6

Table 2 (cont.)

Nome Popular (Nome Científico)	Origem do Fornecedor	Partes analisadas	Rutina (mg.Kg^{-1})		
			Média	DP	Média entre fornecedores
Vagem Carnuda (*Lablab purpureus (L) Sweet*)	1 1 3	Semente,vagem	- - -		-
Rabanete (*Raphanus sativus L.*)	1 1 1	Casca,raiz	- - -		-
Alecrim (*Rosmarinus officinalis L.*)	1 1 1	Folha	- - -		-
Chicória/Escarola (*Cichorium endivia L.*)	1 1 1	Folha	- - -		-
Couve manteiga (*Brassica oleracea L. var. acephala D.C*)	1 1 1	Folha	- - -		-
Espinafre (*Spinacea oleracea L.*)	1 1 1	Folha	1,4 Nq -	0,1 nq	0,5±0,8
Louro (*Laurus nobilis L.*)	1 1 1	Folha	- - -		-
Orégano (*Origanum vulgare*)	1 1 1	Folha	- - -		-
Salsão/Aipo (*Apium graveolens*)	1 1 1	Folha	- - -		-
Sálvia (*Salvia officinalis*)	1 1	Folha	- -		-
Acelga (*Beta vulgaris L. var. cicla*)	1 1 1	Folha,talo	4,4 [b] 7,1 [a] 1,1 [c]	0,1 1,2 0,2	4,2±3,0
Agrião (*Nasturtium officinale sp.*)	1 1 1	Folha,talo	3,0 [a] 4,2 [a] -	0,3 0,7	2,4±2,2
Alface crespa (*Lactuca sativa L.*)	1 1 1	Folha,talo	- - -		-
Alface roxa (*Lactuca sativa L.*)	1 1 1	Folha,talo	- - -		-
Almeirão (*Cichorium intybus L*)	1 1 1	Folha,talo	- - -		-
Aspargo (*Asparagus officinalis L.*)	13 10 10	Folha,talo	48,4 [c] 233,3[a] 172,1[b]	3,4 8,4 4,6	151,3±94,2

Table 2 (cont.)

Nome Popular (Nome Científico)	Origem do Fornecedor	Partes analisadas	Rutina (mg.Kg^{-1})		
			Média	DP	Média entre fornecedores
Cebolinha (*Allium schoenoprasum L.*)	1	Folha,talo	10,5 ^{a}	0,9	7,4±2,8
	1		6,2 ^{b}	0,4	
	1		5,3 ^{b}	1,0	
Coentro (*Coriandrum sativum L.*)	1	Folha,talo	58,3 ^{b}	6,0	196,6±245,1
	1		51,9 ^{b}	6,3	
	1		479,6^{a}	3,7	
Manjericão (*Ocimum basilicum L.*)	1	Folha,talo	20,3 ^{b}	3,4	27,9±8,3
	1		26,7^{ab}	2,6	
	1		36,7 ^{a}	2,7	
Mostarda (*Brassica juncea (L.) Coss*)	1	Folha,talo	23,0 ^{a}	0,8	24,1±1,5
	1		25,1 ^{a}	0,2	
Repolho (*Brassica oleracea L./ Brassica oleracea var. capitata ''f.alba''*)	1	Folha,talo	1,1 ^{b}	0,1	2,0±1,6
	1		1,0 ^{b}	0,2	
	1		3,9 ^{a}	0,1	
Repolho Roxo (*Brassica oleracea L./ Brassica oleracea var. capitata ''f.rubra''*)	1	Folha,talo	-		-
	1		-		
	1		-		
Rúcula (*Eruca sativa L.*)	1	Folha,talo	-		-
	1		-		
	1		-		
Salsa (*Petroselinum crispum (Mill.) Nym*)	1	Folha,talo	-		-
	1		-		
	1		-		
Alho-poró (*Allium ampeloprasum L.*)	1	Talo	1,9 ^{ab}	0,4	3,3±1,2
	1		4,3 ^{a}	0,4	
	1		3,6 ^{b}	0,4	
Couve-Flor (*Brassica oleracea var. botritys*)	1	Talo,flor	9,9 ^{a}	0,2	11,9±2,6
	1		14,9 ^{a}	2,0	
	1		11,0 ^{a}	1,4	
Brócolis ninja (*Brassica oleracea L. var. italica Plenck*)	1	Talo,flor	18,4 ^{b}	2,2	16,9±9,9
	1		6,3 ^{c}	1,0	
	1		26,0 ^{a}	1,4	
Broto de Feijão (*Vigna radiata*)	1	Broto	29,5 ^{b}	1,7	37,4±9,4
	1		34,9 ^{b}	2,9	
	1		47,8 ^{a}	1,0	
Batata doce rosada (*Ipomoea batatas L.*)	1	Raiz	0,9	0,2	0,3±0,5
	1		-		
	1		-		
Beterraba (*Beta vulgaris L.*)	1	Raiz	-		-
	1		-		
	1		-		
Cenoura (*Daucus carota L.*)	1	Raiz	-		-
	1		-		
	1		-		
Mandioca (*Manihot esculenta Crantz*)	1	Raiz	2,4 ^{b}	0,0	2,6±1,7
	1		4,3 ^{a}	0,2	
	1		1,0 ^{c}	0,0	

Table 2 (cont.)

Nome Popular (Nome Científico)	Origem do Fornecedor	Partes analisadas	Rutina (mg.Kg^{-1})		
			Média	DP	Média entre fornecedores
Mandioquinha (*Arracacia xanthorrhiza Banc.*)	1	Raiz	4,3 ^{a}	0,6	2,9±1,2
	1		2,0 ^{b}	0,4	
	1		2,5 ^{b}	0,1	
Nabo (*Brassica rapa var. rapa (L.) Thell.*)	1	Raiz	0,7 ^{c}	0,0	1,6±1,1
	1		2,9 ^{a}	0,1	
	1		1,2 ^{b}	0,1	
Gengibre (*Zingiber officinale Roscoe*)	1	Rizoma	3,8 ^{b}	0,2	4,1±0,4
	1		3,9 ^{ab}	0,5	
	1		4,6 ^{a}	0,3	
Inhame roxo (*Colocasia esculenta L. Schott*)	1	Rizoma	4,8 ^{b}	0,9	5,7±1,4
	1		5,0 ^{b}	0,5	
	1		7,3 ^{a}	0,2	
Batata inglesa (*Solanum tuberosum ssp. Tuberosum*)	3	Tubérculo	2,7 ^{a}	0,4	2,0±0,9
	3		1,0 ^{b}	0,2	
	3		2,3 ^{ac}	0,1	
Cará (*Dioscorea alata L.; Dioscorea rotundata Poir; Dioscorea cayenensis*)	1	Tubérculo	1,0 ^{a}	0,0	0,8±0,2
	1		0,6 ^{b}	0,0	
	1		0,8 ^{ab}	0,1	
Alho roxo (*Allium sativum L.*)	11	Bulbo	-		2,1±2,1
	11		4,2 ^{a}	0,1	
	11		2,2 ^{b}	0,2	

Resultados de análises em triplicata; Origem das amostras: 1) Brasil (Sudeste); 2) Brasil (Norte); 3) Brasil (Sul); 4) Brasil (Nordeste); 5) Colômbia; 6) Brasil (Centro-Oeste); 7) Portugal; 8) Espanha; 9) Estados Unidos; 10) Chile; 11) Argentina; 12) México; 13) Peru; DP: desvio padrão relativo; - : não detectado; nq: abaixo do limite de quantificação; As amostras foram identificadas quanto ao nome científico e popular conforme os dados da Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA); Média entre fornecedores considerando nq=limite de detecção. Letras diferentes ao lado da média indicam que há diferença entre as concentrações, segundo o teste de Tukey, com 95% de confiança.

3.3. TEOR DE RUTINA EM PORÇÕES HABITUAIS DE CONSUMO

Os dados adquiridos foram relacionados com porções de cada vegetal para verificar o potencial de contribuição da rutina como fonte de composto bioativo na dieta humana. Considerando a quantidade que cada vegetal é consumido (porção em uma refeição) pode se verificar que a massa de laranja ou banana consumida será maior que a massa de alho, manjericão ou coentro, por exemplo. Nesse intuito, na Figura 1 foram apresentadas estimativas das porções que o consumidor ingere em uma refeição e, o teor de rutina médio da porção.

Anteriormente, quando observado apenas o teor de rutina presente na planta, as amostras como o coentro, o broto de feijão e o manjericão apresentavam concentrações acentuadas (196,6, 37,4 e 27,9 mg.kg^{-1}, respectivamente). No entanto, quando considerada a

quantidade ingerida pelo consumidor em uma porção, os valores são de apenas 0,1, 1,9 e 0,01 mg de rutina, respectivamente, já que são amostras que se consomem em pequenas quantidades (1,0, 50,0 e 1,0 g, respectivamente).

Sob o cenário da estimativa de ingestão por porção consumida, os vegetais que possibilitam a maior ingestão de rutina são: 200 g de umbu (32,5 mg/porção), 200 g de noni (19,8 mg/porção); 100 g de aspargo (15,1 mg/porção), 200 g de amora (12,1 mg/porção), 200 g de marmelo (11,7 mg/porção), 200 g de cereja (8,6 mg/porção) e 200 g de ameixa roxa (5,1 mg/porção).

Estudos reportaram que a rutina apresenta efeitos benéficos a saúde, como anti-hiperglicêmico, em doses a partir de 5,0 mg por kg de massa corpórea por dia (GHORBANI, 2017). Sendo assim, para um adulto de 70 kg é necessária uma ingestão mínima de aproximadamente 350,0 mg de rutina, embora este valor possa variar para cada indivíduo de acordo com seu metabolismo e a biodisponibilidade da rutina (LESSER; KEEN; LANOUE, 2015).

Quando comparadas as porções de frutas e hortaliças com outros alimentos presentes na dieta, observa-se que existe uma gama extensa de fontes desse flavonóide, o que possibilita o seu consumo para pessoas que apresentem preferências diversificadas. Uma porção (240 mL) de chá verde ou preto, preparada com 2,0 g de planta, apresenta, em média, 11,9 mg de rutina (JESZKA-SKOWRON; KRAWCZYK; ZGOŁA-GRZEŚKOWIAK, 2015). Essas quantidades seriam alcançadas ao ingerir 72,9 g de umbu, 78,5 g de aspargo, 195,9 g de amora e 206,0 g de marmelo. Segundo Fratianni et al. (2014), a mesma quantidade está presente em 177,3 g de lentilha.

Levando em consideração que uma dieta balanceada é composta de diferentes alimentos e bebidas é possível atingir a quantidade mínima de rutina para que o organismo apresente benefícios à saúde.

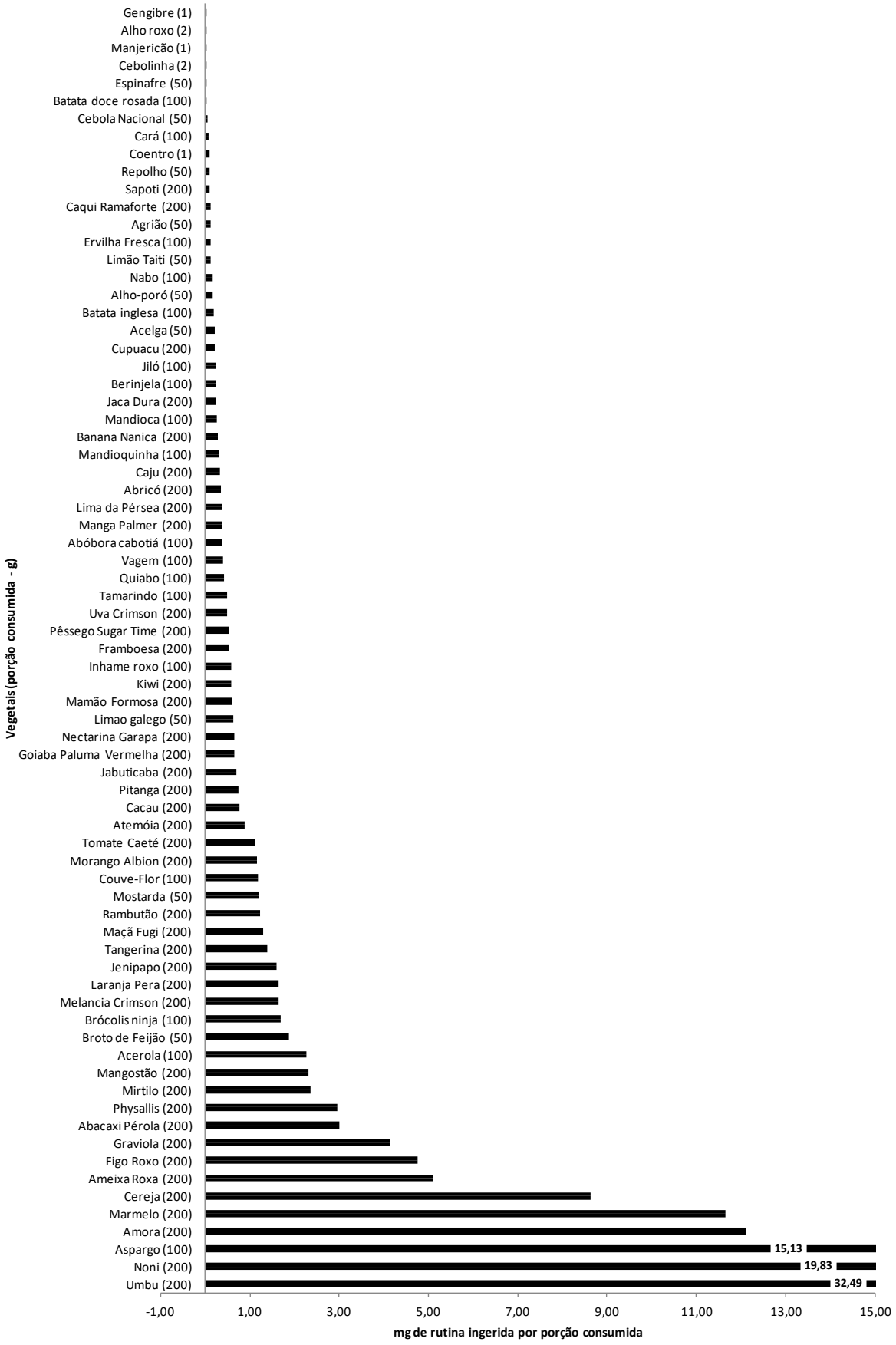

Figura 1 Estimativa da quantidade média de rutina ingerida por porção consumida

4. CONCLUSÃO

Esse estudo permitiu a determinação do conteúdo de rutina em 117 vegetais que são comercializados no Brasil, detectando sua presença em mais de 60% das amostras. Os vegetais que se destacaram pela elevada concentração de analito foram o coentro, umbu, aspargo, noni, amora, marmelo e cereja, com concentração média variando de 43,2 a 196,6 $mg.kg^{-1}$. Quanto consideradas as porções normalmente ingeridas dos vegetais analisados, é possível observar que as frutas e hortaliças se aproximam de outras fontes de rutina, sendo que as frutas que apresentaram maior quantidade foram umbu, noni, amora, marmelo, cereja e ameixa roxa. Dentre as hortaliças, o aspargo apresentou maior teor. Dessa forma, evidencia-se que é possível, a partir de uma dieta diversificada, ter a ingestão de rutina em quantidades que apresentem atividade biológica, proporcionando benefícios à saúde humana.

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DISCUSSÃO GERAL

Pessoas que buscam uma vida mais saudável vem procurando cada vez mais a inclusão de alimentos que possuem constituintes bioativos, já que vários estudos os correlacionam com a prevenção de doenças como diabetes, câncer e obesidade (KREMR et al., 2015; SIRIAMORNPUN; KAEWSEEJAN, 2017).

Frutas e hortaliças, além de serem fontes de diversos macro e micronutrientes, apresentam em sua composição várias classes de compostos bioativos, como os compostos fenólicos, carotenoides e tocoferóis. Essas substâncias possuem funções fisiológicas e metabólicas específicas, as quais já foram relacionadas à prevenção de diversas doenças crônicas (BARBA et al., 2017; JESZKA-SKOWRON; STANISZ; DE PEÑA, 2016). Dentre as classes de compostos fenólicos existentes destacam-se várias como os flavonoides – dos quais a rutina é um representante – os ácidos clorogênicos e o ácido cafeico.

Nesse contexto, os ácidos clorogênicos são compostos fenólicos que tem como principais fontes, já descritas na literatura científica, o café e chás (BUDRYN; ZACZYŃSKA; ORACZ, 2016; CRAIG et al., 2016; DA SILVEIRA et al., 2016). Os benefícios à saúde, relacionados a essa classe vêm sendo estudado por diversos pesquisadores que chegaram à conclusão de que há um impacto positivo na prevenção de doenças (HUANG et al., 2017b; MILLS et al., 2016; SISWANTO; OGURO; IMAOKA, 2017). Entretanto, não existem estudos extensos acerca do conteúdo de ácidos clorogênicos em frutas e hortaliças, o que deixa em aberto um potencial de descoberta para novas fontes dessas substâncias.

O Brasil se encontra entre os maiores produtores de frutas e hortaliças, se encontrando em terceiro lugar no ranking mundial quando se trada de frutas, atrás apenas da China e da Índia. As exportações de frutas constituem uma porcentagem significativa em relação a outros setores, sendo que as espécies mais comercializadas com outros países são melão, manga, limão e banana, todos cultivos já estabelecidos pelos produtores brasileiros (SEBRAE, 2015). No entanto, a vasta biodiversidade do país, conta com uma grande quantidade de espécies vegetais que ainda não tem seu potencial inteiramente explorado, o que gera falta de interesse em sua produção (SCHIASSI et al., 2018).

O conhecimento do conteúdo de compostos bioativos, como os ácidos clorogênicos, ácido cafeico e rutina em frutas e hortaliças ajuda a agregar valor e promover interesse em espécies que podem vir a ser fontes potenciais dessas substâncias, inclusive sugerindo alternativas às fontes tradicionais já conhecidas na ciência. Dessa forma, este trabalho buscou identificar e quantificar o ácido cafeico, rutina e isômeros de ácidos clorogênicos em frutas e

hortaliças, de diferentes fornecedores, comercializadas no Brasil utilizando cromatografia líquida de alta eficiência (HPLC) acoplada a um detector de arranjo de diodos.

Foram feitas análises de umidade, sólidos solúveis totais (SST) e acidez total titulável (ATT) em todas as amostras. A medição de SST e ATT é importante para avaliar as características pós colheita das frutas e hortaliças e são usadas para fazer uma inferência em relação ao nível de maturação (NOOKARAJU et al., 2010). Entre as frutas, 93% das amostras apresentaram umidade entre 70% e 97%, tendo apenas o tamarindo e o jatobá se destacando com umidades bem inferiores (10% e 21% respectivamente). Já para as hortaliças das 153 amostras, 131 apresentaram umidade maior que 80,0%, 17 amostras entre 61,1 e 78,0% e 5 amostras entre 37,8 e 55,9%. As amostras de louro e pepino tiveram o menor e o maior teor de umidade, com valores médios de 40,8% e 96,2%, respectivamente.

Em relação aos resultados de sólidos solúveis totais, os valores variaram entre 1,4ºBrix e 51ºBrix para as frutas e 1,2ºBrix e 34,0ºBrix para as hortaliças. Para a ATT, a maior parte das amostras de frutas apresentaram valores entre 0,03% e 2,1% com exceção do cupuaçu, maracujá, limão, lima e tamarindo que chegaram ficaram entre 2,8% e 12,2%. Os valores de acidez para as hortaliças foram inferiores aos das frutas, variando entre 0,03% e 0,47%, sendo que os maiores valores foram das amostras de alho roxo, broto de feijão e salsa. Os maiores ratios entre as frutas foram encontrados no coco, abiu, pinha, caqui, abricó, sapotilha e cana-de-açúcar, variando entre 130 e 550 e entre as hortaliças na abóbora cabotiá, louro e cenoura (entre 100,8 e 241,4). Os valores encontrados corroboram com os resultados verificados em outros estudos (CANET et al, 2016; GHOLAMI, 2011).

O método utilizado para a identificação e quantificação dos compostos bioativos foi baseado em Meinhart, et al (2017) e validado para os parâmetros de limites de quantificação e identificação, linearidade, precisão no dia e entre dias e exatidão. Para todos os compostos, as curvas analíticas apresentaram linearidade adequada, com os valores de F, para falta de ajuste, inferiores ao valor de F crtítico, para 95% de confiança. Os limites de detecção ficaram entre 0,005 $mg.kg^{-1}$ e 0,008 $mg.kg^{-1}$ e os limites de quantificação foram de 0,01 $mg.kg^{-1}$. Os valores de precisão foram inferiores a 10% para todos os níveis e em relação à exatidão do método, os níveis de recuperação variaram, para os três níveis de concentração, entre 91,7 e 108,1%. Os parâmetros estão de acordo com os limites estabelecidos pela IUPAC (THOMPSON; ELLISON; WOOD, 2002) e pela ANVISA (2017), demonstrando que o método apresentou requisitos analíticos satisfatórios para realizar análises quantitativas das amostras.

Seis isômeros de ácidos clorogênicos foram identificados e quantificados em frutas e hortaliças. O 3-CQA foi encontrado em 5 frutas com concentrações variando de 0,47 $mg.kg^{-1}$

a 199,14 mg.kg^{-1}, sendo que as frutas com maiores valores foram a cereja, marmelo e abiu. O estudo realizado por Bastos et al. (2015) em cereja quantificou esse composto, encontrando resultados similares ao verificado nesse estudo (135,50 mg.kg^{1} a 199,10 mg.kg^{1}). Não foram encontrados trabalhos que quantificaram o 3-CQA em abiu, enquanto nessa análise foi encontrado um valor entre 0,80 mg.kg^{-1} and 21,30 mg.kg^{-1} evidenciando a variação entre os fornecerdores, o que pode estar relacionado a diferenças entre cultivares, praticas e cultivação, solo e condições climáticas (RICKMAN; BRUHN; BARRET, 2007). Das 64 frutas analisadas, 44 não possuíam estudos prévios sobre a investigação do conteúdo de 3-CQA.

Em relação às hortaliças, o 3-CQA foi encontrado apenas em 3 amostras, sendo elas couve manteiga, quiabo e cenoura, com valores médios entre os fornecedores de 91,1 mg.kg^{-1}, 10,1 mg.kg^{-1} e 0,3 mg.kg^{-1}, respectivamente. Fiol et al. (2012) realizaram a identificação do 3-CQA em couve manteiga sem, no entanto, quantificá-lo. As outras amostras onde esse analito foi detectado não apresentam estudos que o reportem.

Entre os isômeros analisados, o 5-CQA é o ácido clorogênico mais reportado na literatura em vegetais (BARROS et al., 2012; YOON; CHUNG; THIRUVENGADAM, 2015) e foi encontrado em 55 amostras de frutas (com valores entre 0,19 mg.kg^{-1} and 522,33 mg.kg^{-1}) e também 55 amostras de hortaliças (concentrações entre 0,01 e 128,0 mg.kg^{-1}). As frutas apresentaram teores mais elevados desse composto em relação às hortaliças e as que se destacaram foram o tangerina, abiu, jaca, nectarina, marmelo, mirtilo and amora, valores superiores ao encontrado por Gungdogdu et al. (2011) em mirtilo e Pontes et al. (2002) em jaca. A tangerina e outras 17 frutas não tiveram o conteúdo de 5-CQA reportado em outros estudos. O almeirão, alface roxa, salsão e berinjela, apresentaram os maiores teores de 5-CQA com valores médios entre fornecedores, respectivamente, de 80,9, 76,6, 52,5 e 48,5 mg.kg^{-1}.

No que concerne à identificação e quantificação do 4-CQA, apenas 19 frutas possuíam estudos prévios na literatura enquanto, nesse trabalho, o composto esteve presente em 22 frutas com as maiores concentrações em morango (12,02 mg.kg^{-1} a 80,25 mg.kg^{-1}), amora (29,87 mg.kg^{-1}) e tamarindo (6.26 mg.kg^{-1} a 13,39 mg.kg^{-1}), Já nas hortaliças, a presença do 4-CQA foi detectada em 11 vegetais, variando entre 0,1 e 38,2 mg.kg^{-1}, sendo que as amostras de aspargo (32,2 mg.kg^{-1}), cebolinha (13,7 mg.kg^{-1}) e manjericão (12,8 mg.kg^{-1}) se mostraram com os maiores teores médios. Nenhuma dessas amostras possui dados reportados na literatura sobre a identificação e quantificação deste composto.

Em relação aos ácidos dicafeoilquínicos, o 3,4-CQA foi encontrado em 10 frutas (22 amostras) com teores variando entre 0,06 mg.kg^{-1} e 9,08 mg.kg^{-1} e tendo como maiores representantes physalis, cajú, mirtilo e grapefruit. Entre as hortaliças, esse compostos foi

quantificado em 28 amostras com teores entre 0,2 e 190,8 mg.kg^{-1}. As amostras de mostarda, salsão e acelga apresentaram quantidades média superior às demais, com 169,9, 118,7 e 44,0 mg.kg^{-1}, respectivamente. Na literatura, somente foram encontrados trabalhos que relatam a presença do 3,4-DQA para algumas amostras de vegetais. Esatbeyoglu et al. (2016) obtiveram um teor de 109,0 mg.kg^{-1} em batata doce, enquanto apenas a identificação do composto foi feita em almeirão (PAPETTI et al., 2017) e inhame roxo (CHAMPAGNE et al., 2011).

O 3,5-DQA foi encontrado em 26 amostras de 9 frutas e foi o ácido dicafeoilquínico presente no maior número de frutas com teores variando entre 0.25 mg.kg^{-1} e 72.54 mg.kg^{-1}. As amostras de lima, maracujá e kinkan foram os destaques em relação a esse composto, sendo que não existem relatos anteriores na literatura em relação à lima e kinkam, enquanto para o maracujá, apenas um estudo em suco foi desenvolvido por Spínola et al. (2015). As amostras de hortaliças apresentaram um teor de 3,5-DQA maior do que as frutas chegando a um valor médio de 345,9 mg.kg^{-1} para o louro seguido pela rúcula com 26,6 mg.kg^{-1}. Ainda, podem ser citados, com quantidades significativas de 3,5-DQA, o almeirão (15,3 mg.kg^{-1}), alecrim (9,5 mg.kg^{-1}), alface roxa (6,9 mg.kg^{-1}), e batata doce rosa (5,3 mg.kg^{-1}). Dentre as amostras que tiveram teores significativos, a batata doce apresentou estudos prévios por Esatbeyoglu et al. (2016) (266,0 mg.kg^{-1}) e Zheng e Clifford (2008) (apenas identificação por espectrometria de massas), assim como o alecrim, para o qual Meinhart et al. (2017) encontraram 124,0 mg.kg^{-1} em amostras desidratadas.

Quanto ao 4,5-DQA, valores entre 0,08 mg.kg^{-1} ae 535,18 mg.kg^{-1} foram encontrados em 17 amostras de 10 frutas, sendo que a uva, abricó, kinkan e a granadilha apresentaram as maiores concentrações. Entre elas, apenas o abricó foi mencionado em um estudo prévio realizado por Pontes et al. (2002). Já para as hortaliças, os teores de 4,5-DQA foram inferiores, variando entre 0,3 e 62,9 mg.kg^{-1} em 24 amostras. Alecrim, louro, rúcula e orégano estiveram entre as amostras com maiores concentrações médias, de 52,3, 41,3, 27,0 e 9,2 mg.kg^{-1}, respectivamente. Nenhuma dessas amostras possui estudos na literatura que tenham avaliado este composto, exceto para o alecrim onde foi quantificado 8460,6 mg.kg^{-1} em amostras desidratadas, valor superior ao deste estudo (MEINHART et al, 2017).

O ácido cafeico foi quantificado em 9 frutas diferentes, totalizando 20 amostras, e em 22 amostras de hortaliças com concentrações entre 0,23 mg.kg^{-1} e 59,66 mg.kg^{-1} para as frutas e 0,01 e 74,7 mg.kg^{-1} para as hortaliças. As frutas que apresentaram maiores valores médios foram mirtilo (59,66 mg.kg^{-1}), pitaya amarela (10,52 mg.kg^{-1}), kinkan (2,39 mg.kg^{-1}) e cereja (1,61 mg.kg^{-1}),. Já as aamostras de orégano (55,1 mg.kg^{-1}), alecrim (42,2 mg.kg^{-1}), sálvia (41,0 mg.kg^{-1}) manjericão (32,0 mg.kg^{-1}) e coentro (12,9 mg.kg^{-1}) foram as que contiveram

maiores concentrações médias entre as hortaloças. O ácido cafeico não foi investigado anteriormente em orégano, entretanto em manjericão e coentro, esse analito foi apenas identificado por Antora e Salleh (2017), Grădinariu et al. (2013) e El-Zaeddi et al. (2017). No entanto, Meinhart et al. (2017) realizaram a quantificação em sálvia desidratada e Maldini et al. (2016) em alecrim (variando de 34,6 a 720,6 $mg.kg^{-1}$).

Quando considerada a soma de ácido cafeico e clorogênicos, dentre as 107 amostras de frutas que apresentaram quantidades detectáveis, amora, granadilha, marmelo, mirtilo, cereja e morango foram os destaques com concentrações variando entre 57,45 e 535,18 $mg.kg^{-1}$. Em relação às 113 amostras de hortaliça que tiveram alguma quantidade presente desses compostos, o louro, salsão, alecrim, almeirão, couve manteiga, alface roxa, orégano e rúcula apresentaram os maiores somatórios, variando entre 58,5 e 387,2 $mg.kg^{-1}$.

Em relação à rutina, o flavonóide esteve presente em 195 das 324 amostras de frutas e hortaliças analisadas, totalizando 61% do total. Os valores encontrados variaram entre 0,3 e 479,6 $mg.kg^{-1}$, sendo que os vegetais que apresentaram maior quantidade média de rutina entre os fornecedores foram coentro, umbu, aspargo, noni, amora, marmelo e cereja, com concentrações médias de 196,6 $mg.kg^{-1}$, 162,4 $mg.kg^{-1}$, 151,3 $mg.kg^{-1}$, 99,2 $mg.kg^{-1}$, 60,6 $mg.kg^{-1}$,58,3 $mg.kg^{-1}$ e 43,2 $mg.kg^{-1}$, respectivamente. Quando avaliados os 73 vegetais onde a rutina foi encontrada – em um ou mais de seus fornecedores – em 42% deles foi observada uma variação maior que 50% (desvio padrão relativo) na concentração dos diferentes fornecedores.

Embora umbu tenha apresentado um dos resultados mais relevantes em relação a quantificação desse flavoníde, não foram encontrados outros trabalhos que tenham identificado e quantificado rutina nessa fruta. Já para outras amostras como aspargo (SOLANA et al., 2015), noni (LIN et al., 2014; PANDY et al., 2014) e amora (GUNDOGDU et al., 2011) existem referências prévias.

Entre as amostras estudadas 15 frutas não possuíam nenhuma investigação prévia em relação a nenhum dos seis isômeros de ácidos clorogênicos ou o ácido cafeico, (atemoia, cajá manga, pinha, jatoba, kinkan, lima da Pérsia, lima, limão Tahiti, melancia, melão, physalis, pitanga, pitaya amarela, pupunha e tangerina) e, entre as hortaliças, apenas a batata doce, o alecrim, a sálvia e o almeirão já haviam sido reportados em relação a presença de todos esses compostos, demonstrando neste trabalho uma gama considerável de novas informações acerca destes compostos bioativos, bem como a identificação de novas fontes.

CONCLUSÃO GERAL

Este trabalho avaliou as concentrações de oito compostos numa grande variedade de frutas e hortaliças. Em relação à rutina, as amostras que se destacara foram o coentro, umbu, aspargo, noni, amora, marmelo e cereja. Entre as frutas, 67% apresentaram valores quantificáveis de um ou mais isômero de ácidos clorogênicos e cafeico, sendo que o mirtilo e a pitaya foram as melhores fontes de ácido cafeico. Quando considerada a somatória de ácidos cafeoilquínicos (3-CQA, 4-CQA e 5-CQA), os destaques foram o morango, cereja, marmelo e amora, já para os dicafeoilquínicos (3,4-DQA, 3,5-DQA e 4,5-DQA) apresenta-se a kinkan, maracujá, e granadilha.

Com relação às hortaliças avaliadas, as que se destacaram quanto ao somatório dos teores de ácidos monocafeoilquínicos são a couve-manteiga, o almeirão e a alface-roxa, enquanto para o somatório dos dicafeoilquínicos estão o louro, a mostarda e o salsão. Para o ácido cafeico, o orégano, a sálvia e o alecrim se mostraram com as quantidades mais significativas.

Várias das espécies estudadas não possuíam estudos em relação à presença dos seis isômeros de ácidos clorogênicos, ácido cafeico e rutina, demonstrando neste trabalho uma gama considerável de novas informações acerca destes compostos bioativos, bem como a identificação de novas fontes.

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ANEXO

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A3175C7

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A3175C7**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie
Persea americana
Ananas comosus L. Merril
Pouteria caimito
Cucurbita moschata Duch
Mammea american
Euterpe olearacea Mart
Annona cherimola Mill
Persea americana var. Hass e Fuerte
Theobroma cacao
Spondias dulcis Som
Allium cepa L.
Saccharum officinarum
Theobroma grandiflorum